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Kinesin Motor Mechanics: Binding, Stepping, Tracking, Gating, and Limping

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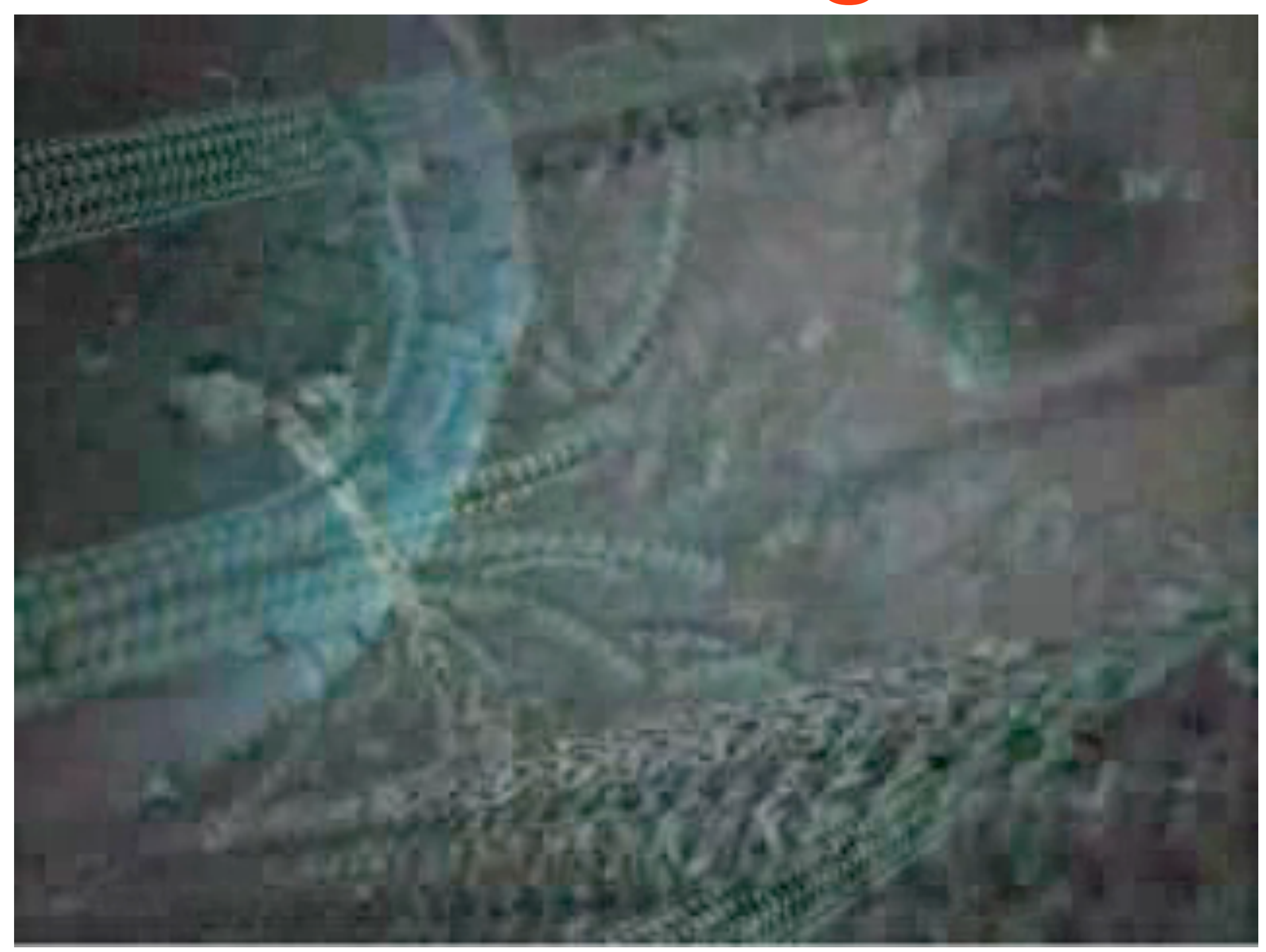
Lecture on a single kinesin



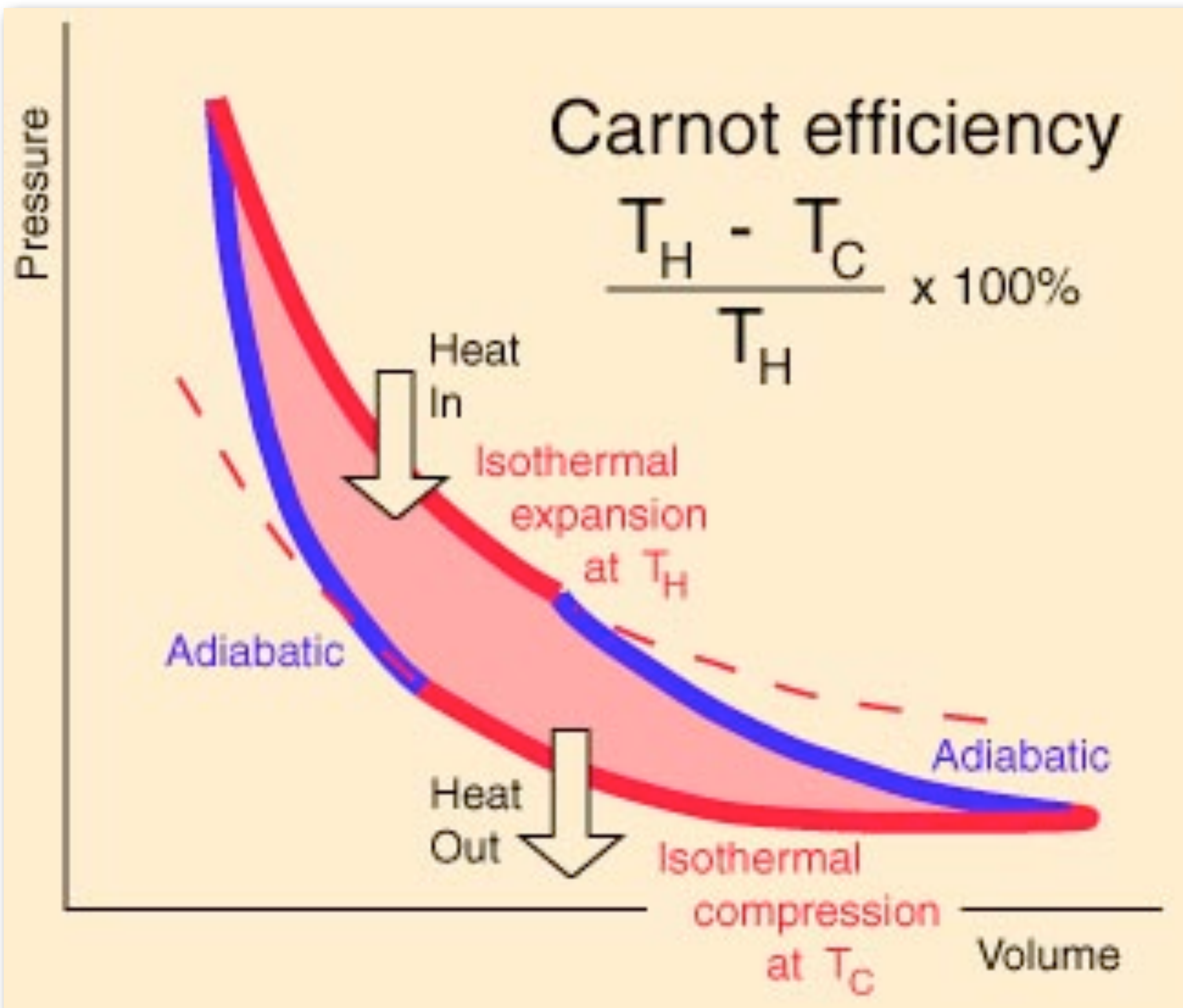
KIAS protein winter school (High1 ski resort, Jan 2011)

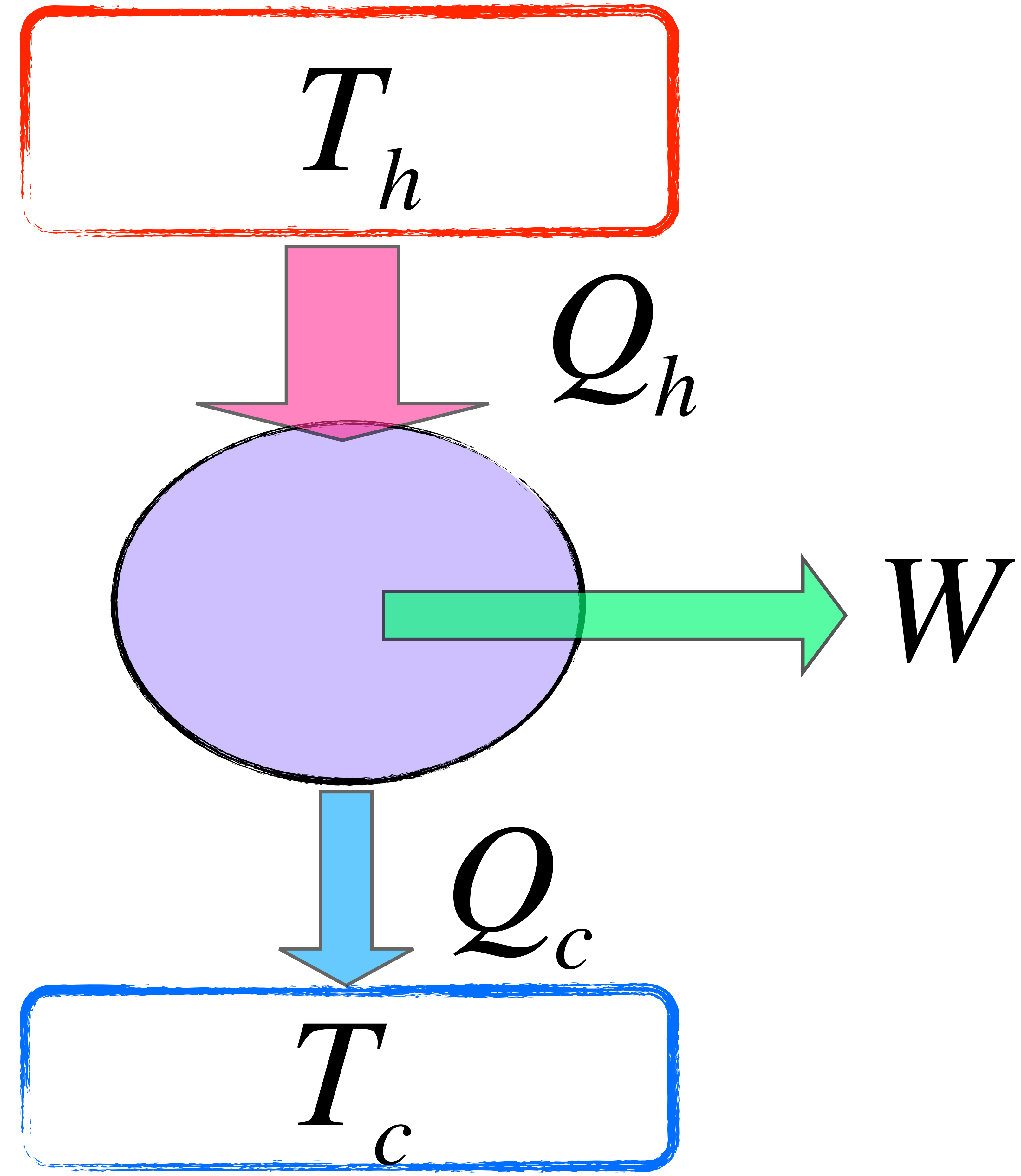
Outlines

- Basics of motors (macroscopic & microscopic engines)
- Basics of kinesin motors (structure, biochemical cycle, dynamics)
- Answering the questions addressed by S. M. Block

Macroscopic engines

Macroscopic heat engine
(Ideal engine)

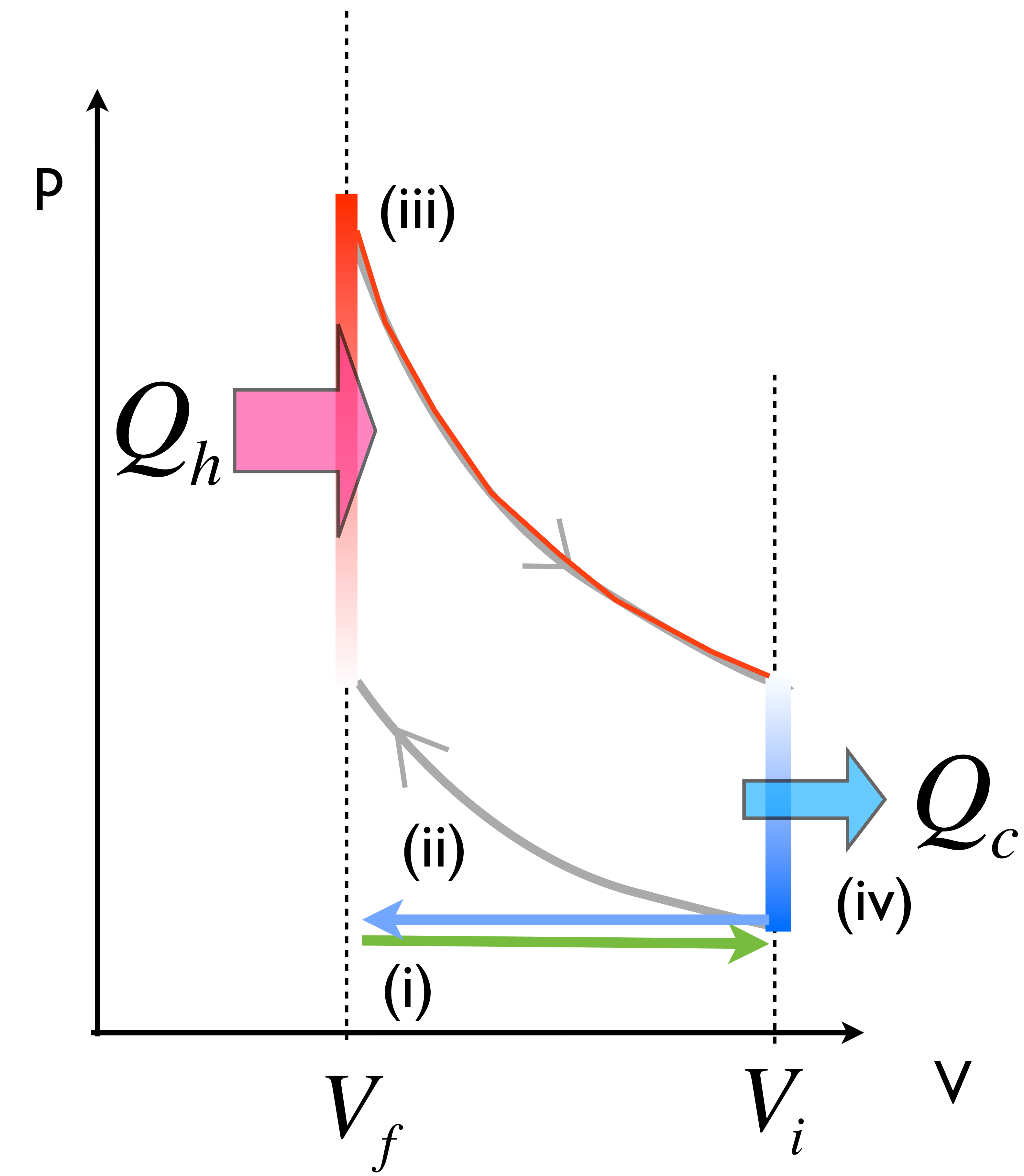




$$-W \leq -W_{\max} = Q_h - Q_c$$

$$\varepsilon < \varepsilon_{\max} = \frac{-W_{\max}}{Q_h} = \frac{Q_h - Q_c}{Q_h} = 1 - \frac{T_c}{T_h}$$

Real engine (four stroke engine)



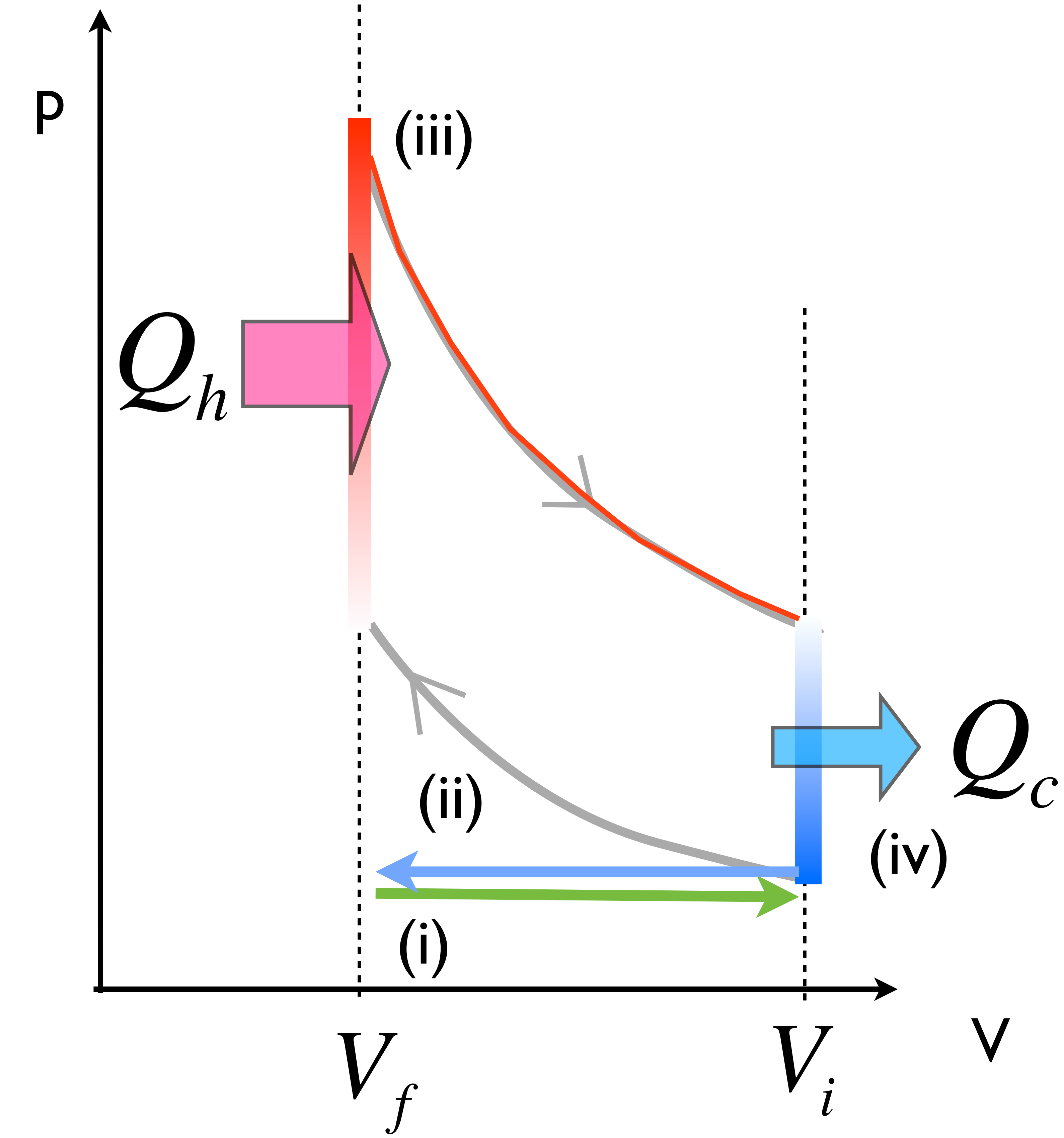
Real engine (four stroke engine)

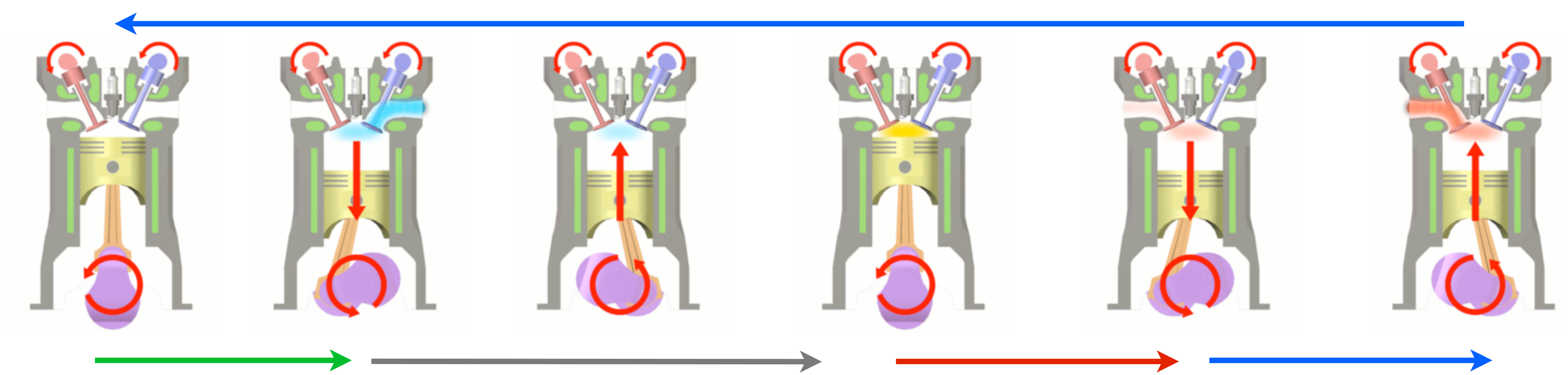
(i) intake stroke performed by an isobaric expansion

(ii) compression stroke performed by an adiabatic compression

(iii) production of isochoric process through the "ignition of fuel", followed by an power stroke (adiabatic expansion)

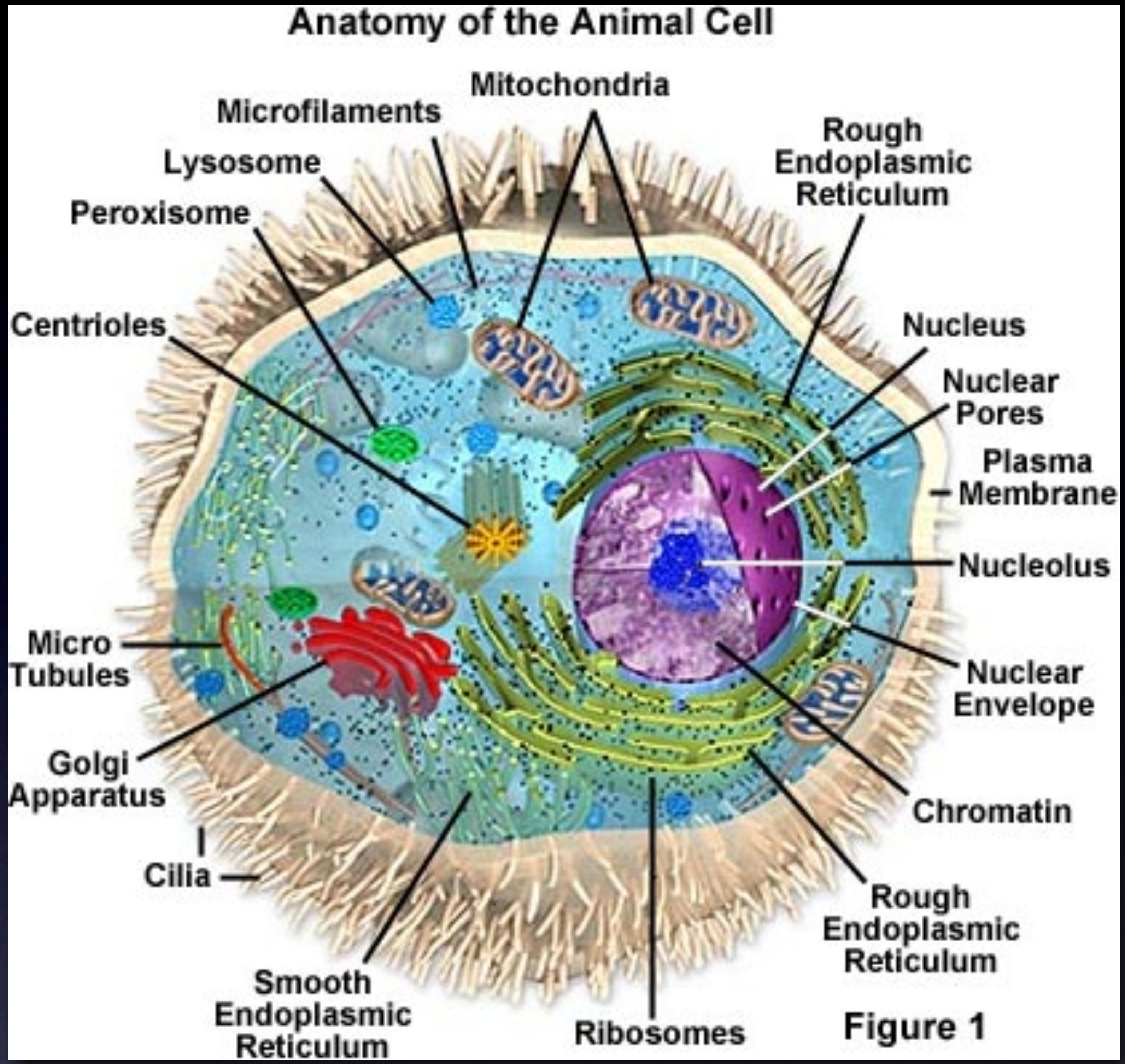
(iv) exhaust stroke by an isochoric and an isobaric compression

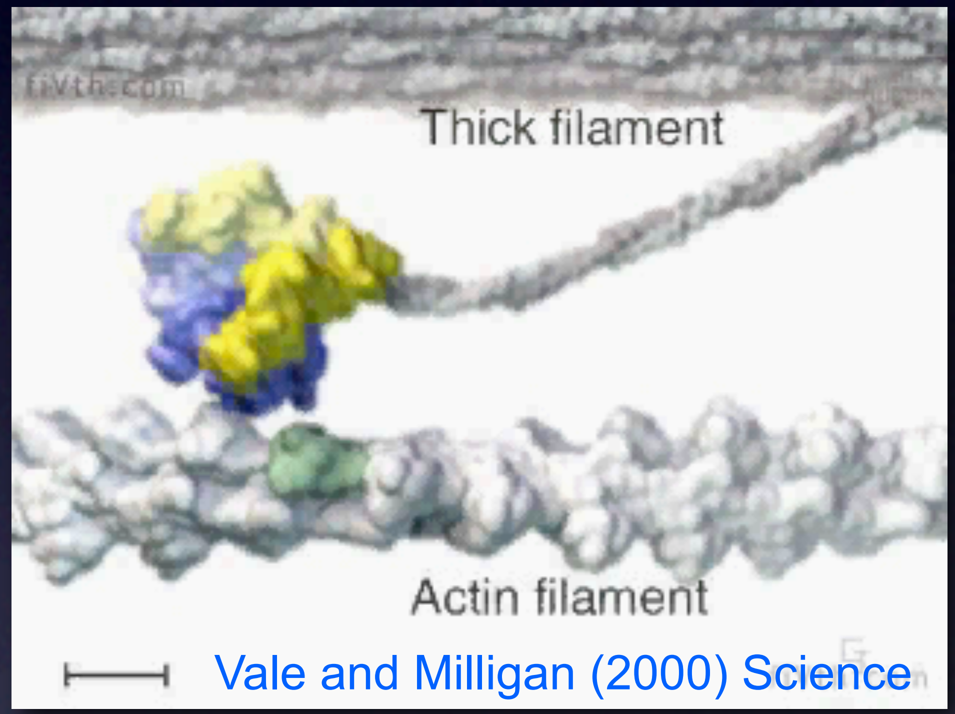


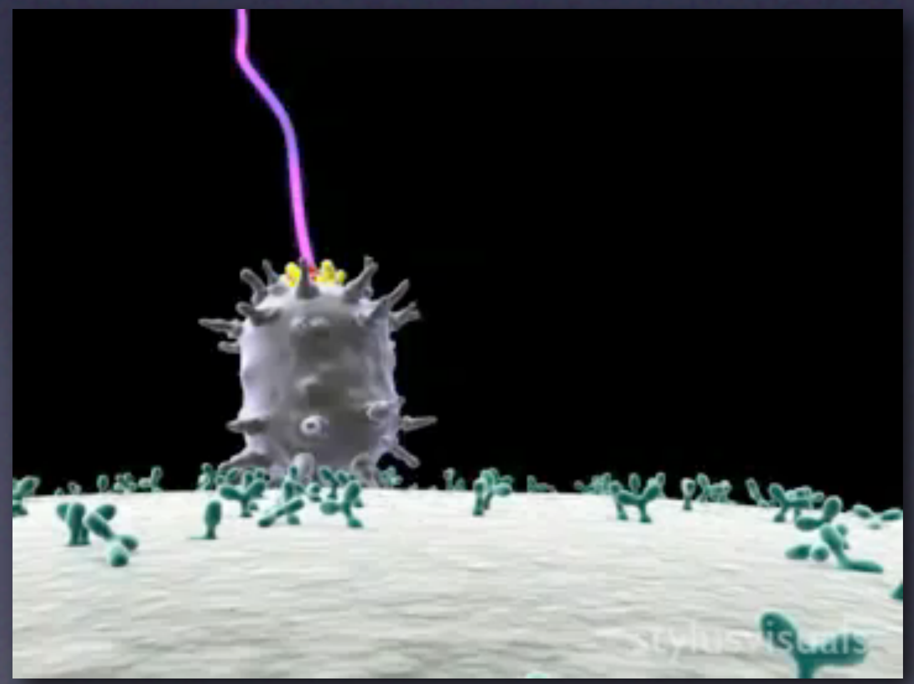


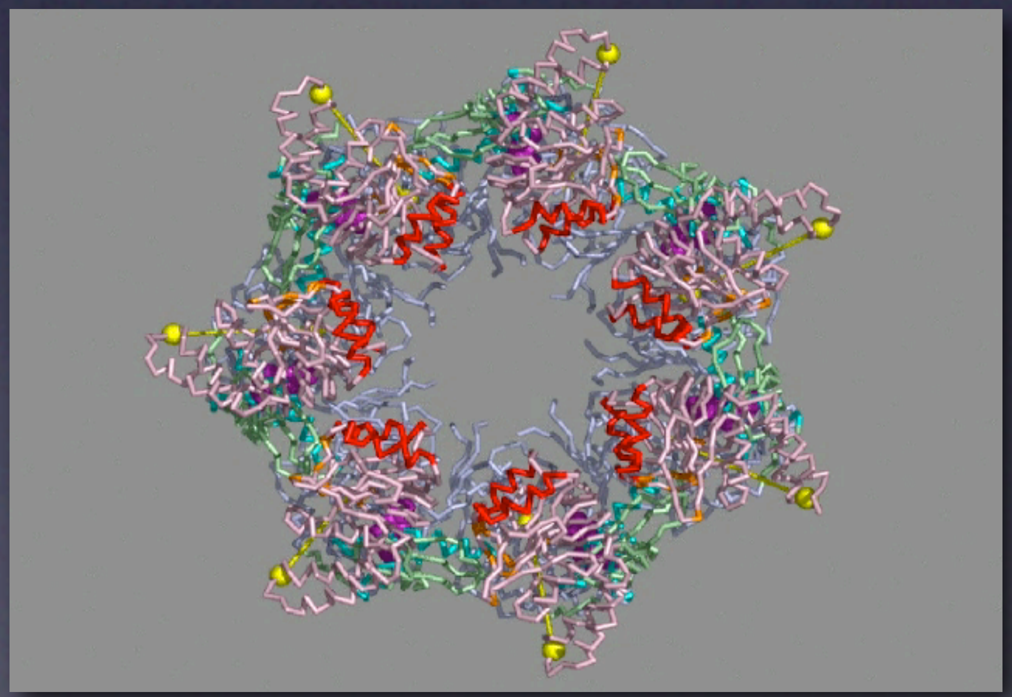
Microscopic engines

Molecular Motors in the Cell

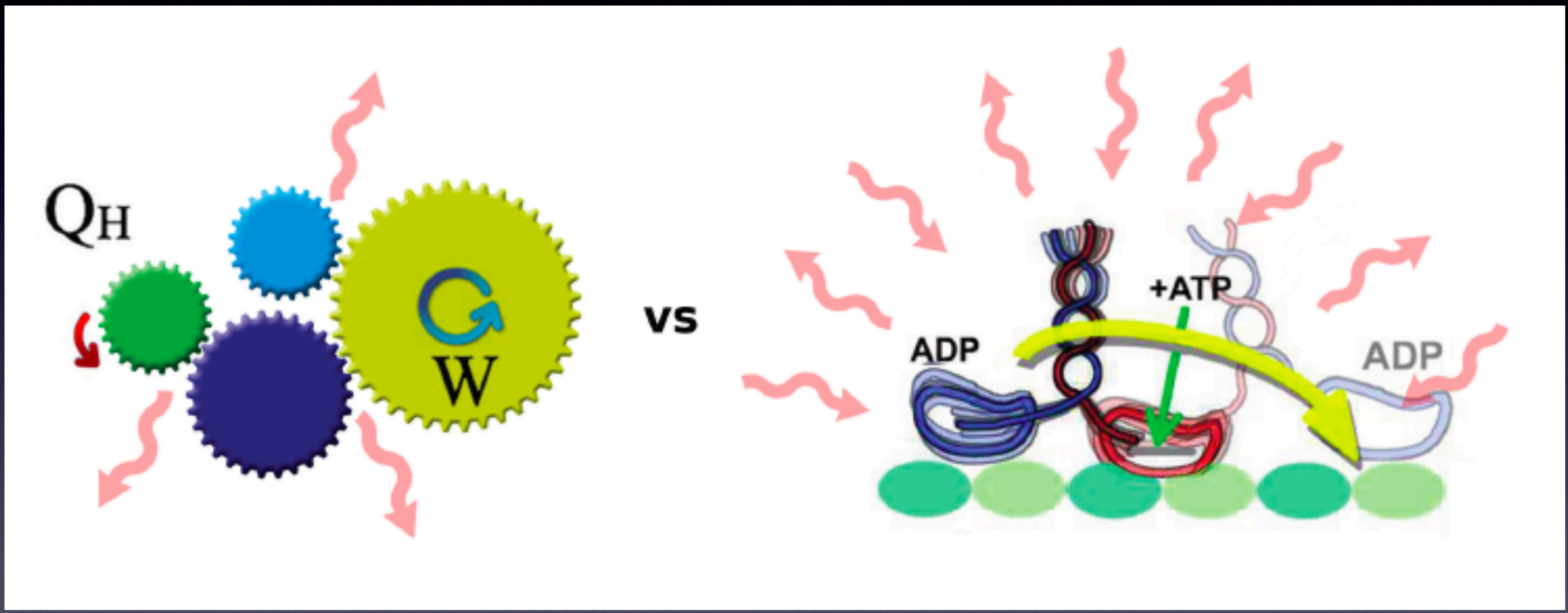








Operational condition for molecular machines



- Size : ~ nm

- Energy scale for noncovalent bond : ~ a few kT. (Covalent bond ~ 200 kT)

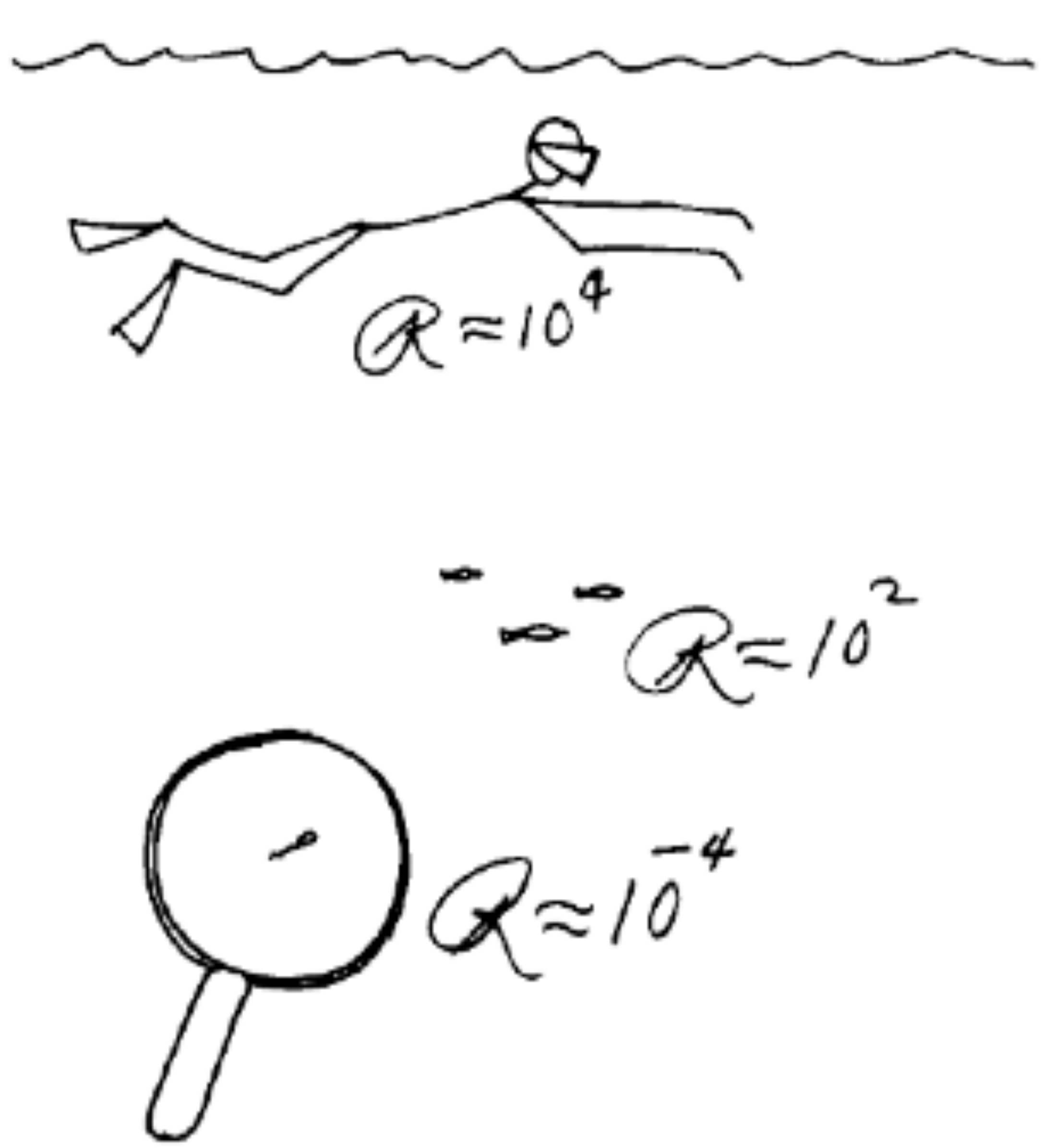
- Overdamped media :

$$\cancel{m\frac{dv}{dt}} = -\zeta v + F \qquad v(t) = v(0)e^{-\frac{\zeta}{m}t} + \frac{1}{m}\int_0^t d\tau e^{-\frac{\zeta}{m}(t-\tau)}F(\tau) \xrightarrow{\frac{\zeta}{m}\gg 1} \frac{F(t)}{\zeta}$$

  - $f_{inertial}/f_{friction} \sim (\rho v L/\eta) = Re << 1$ (ρ:density of object, v: velocity of object, L: size of object, η: viscosity of media)

Dynamics of biomolecules in the cell is dominated by Friction.

Because of different Length, Energy, and Friction scales of biomolecular dynamics in cellular environment, biological motors adopt a fundamentally different strategy from macroscopic machines to perform a work

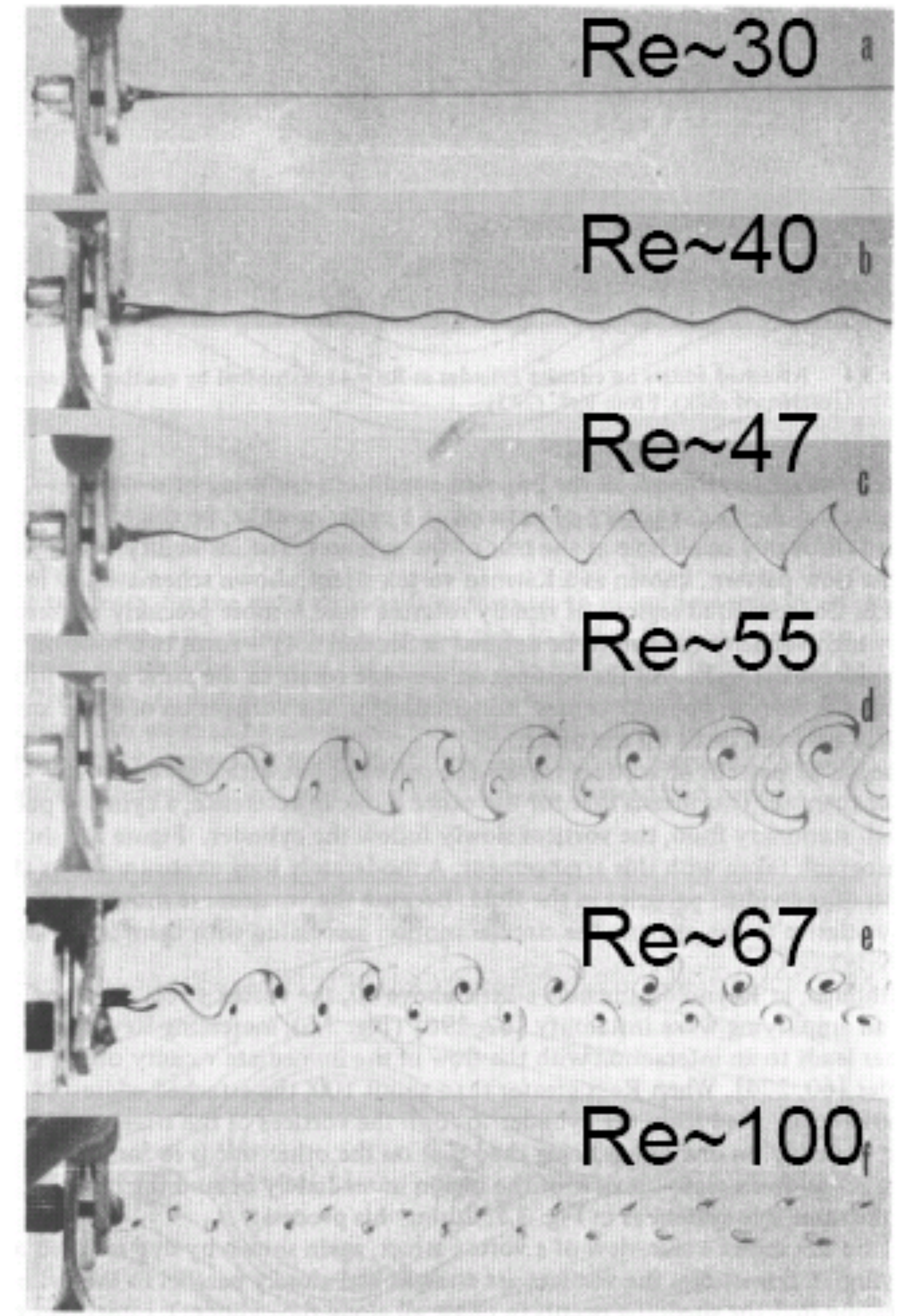


- Reynolds number is dimensionless
- General properties are the same regardless of the geometry
- Re>>1 : high Reynolds number

  Intertial effect dominates, Flow becomes turbulent
- Re<<1 : low Reynolds number

  Damping dominates. Flow: Laminar flow

- Flow patterns become very complex

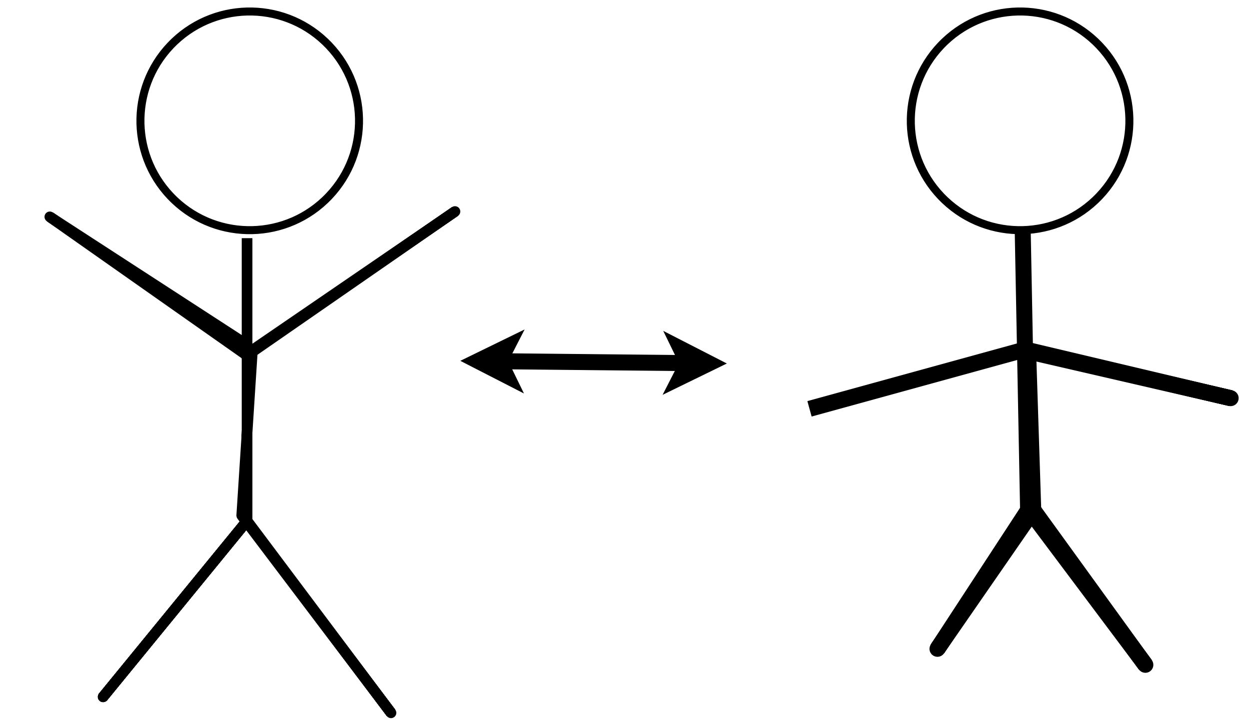


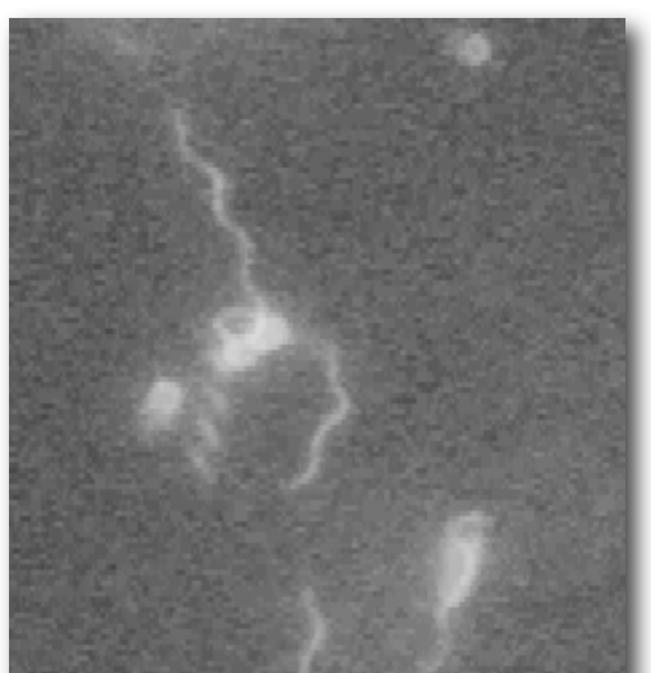
Life in Moving Fluids

	Re
Large whale swimming at 10 ms^{-1}	300,000,000
Duck flying at 20 ms^{-1}	300,000
Large dragonfly going 7 ms^{-1}	30,000
Flapping wings of smallest flying insects	30
Invertebrate larva 0.3mm going at 1 mms^{-1}	0.3
1 μm bacterium swimming at 30 μms^{-1}	0.00003

Increasing effect of viscosity

Need to keep drag down- by design of shape and material characteristics.





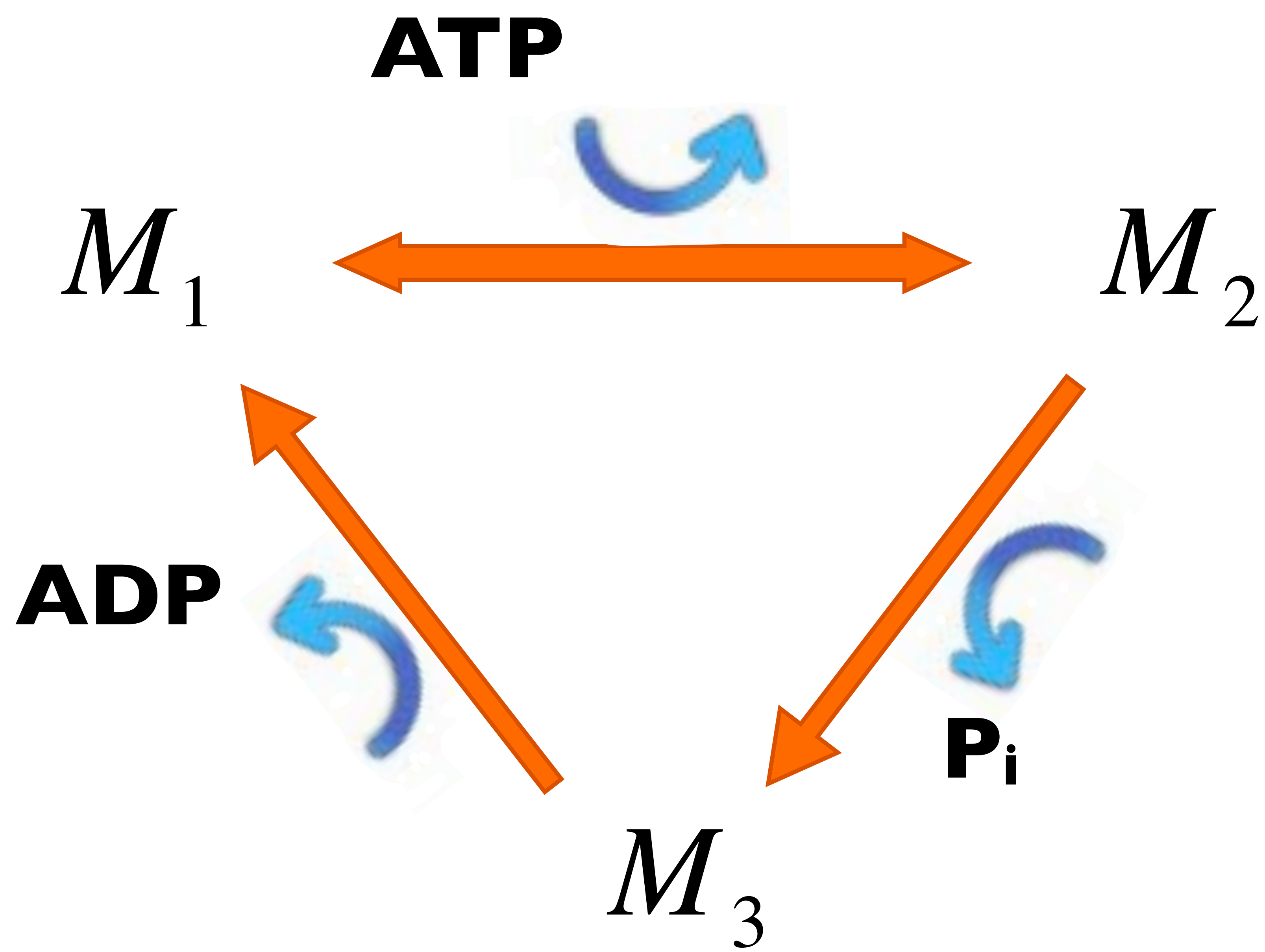
Problem of propulsion – Solved using cilia and flagella, i.e. anisotropic shapes and hence anisotropic drag forces.

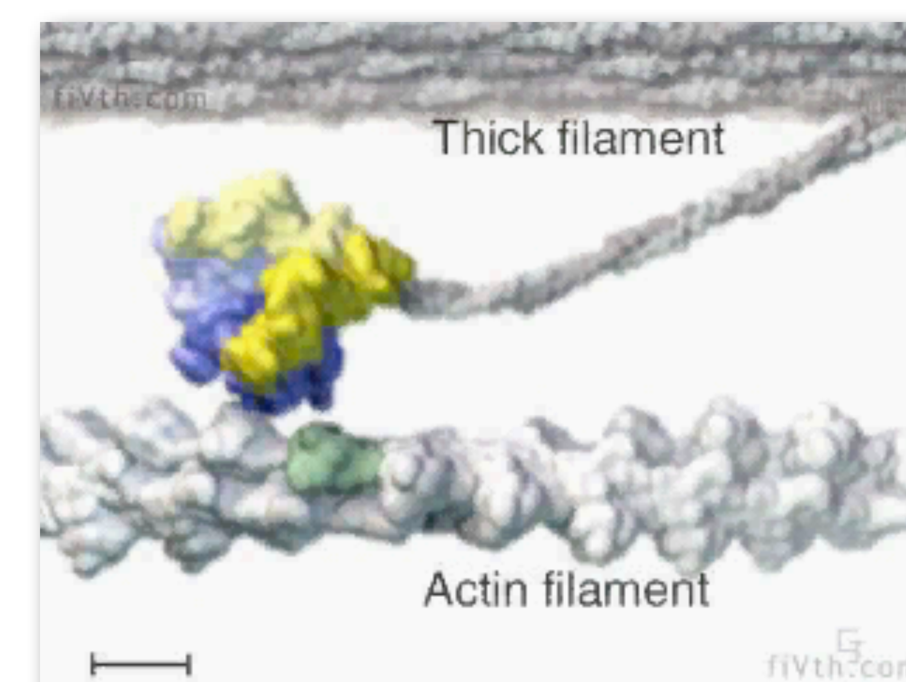
Molecular motors = Enzymes

$$E + S \Leftrightarrow ES \rightarrow E + P$$

$$\underline{M} + ATP \Leftrightarrow \underline{M} \cdot ATP \rightarrow \underline{M} \cdot ADP + P_i \rightarrow \underline{M} + ADP$$

$$M_1 + ATP \Leftrightarrow M_2 \cdot ATP \rightarrow M_3 \cdot ADP + P_i \rightarrow M_1 + ADP$$

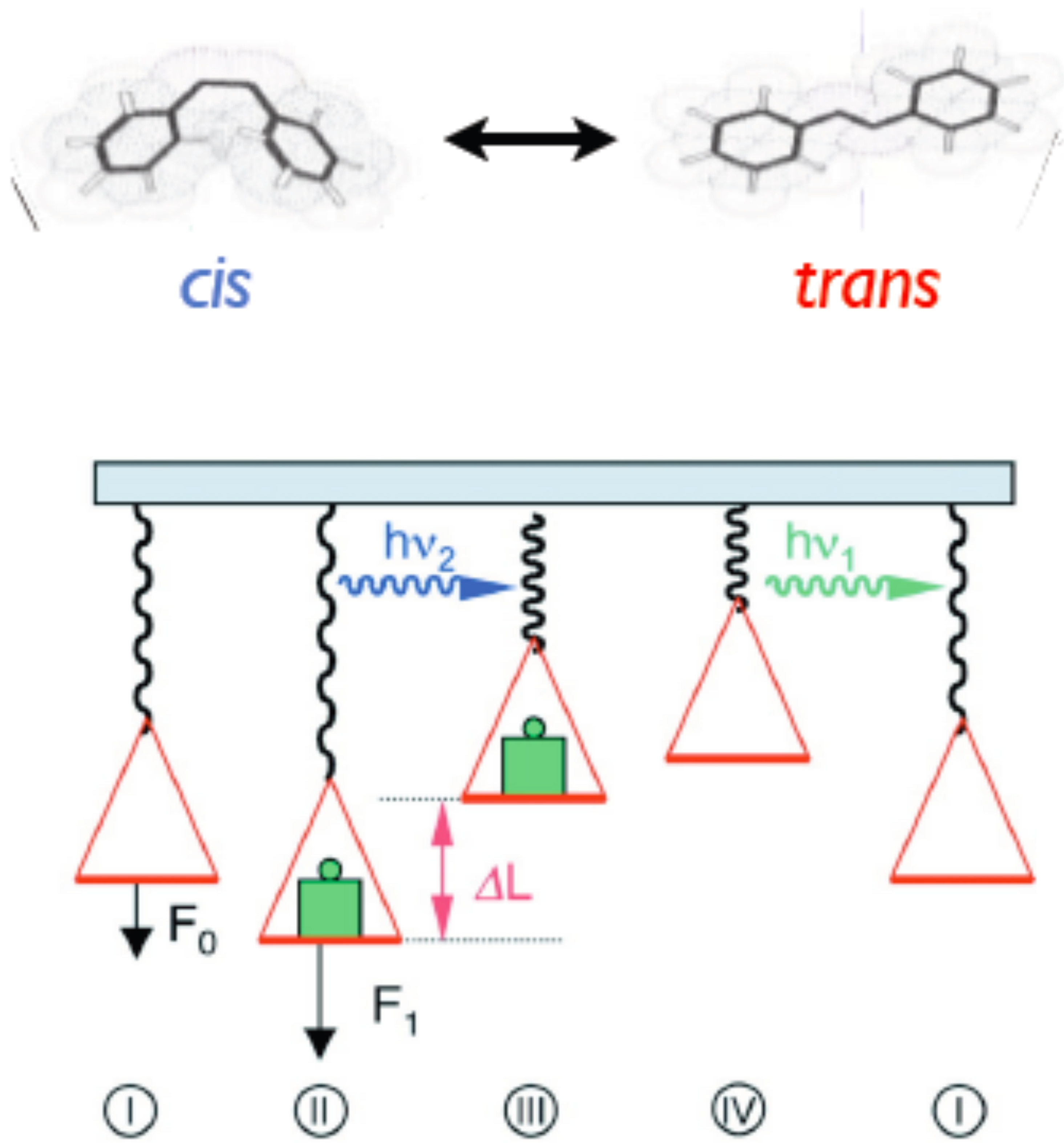


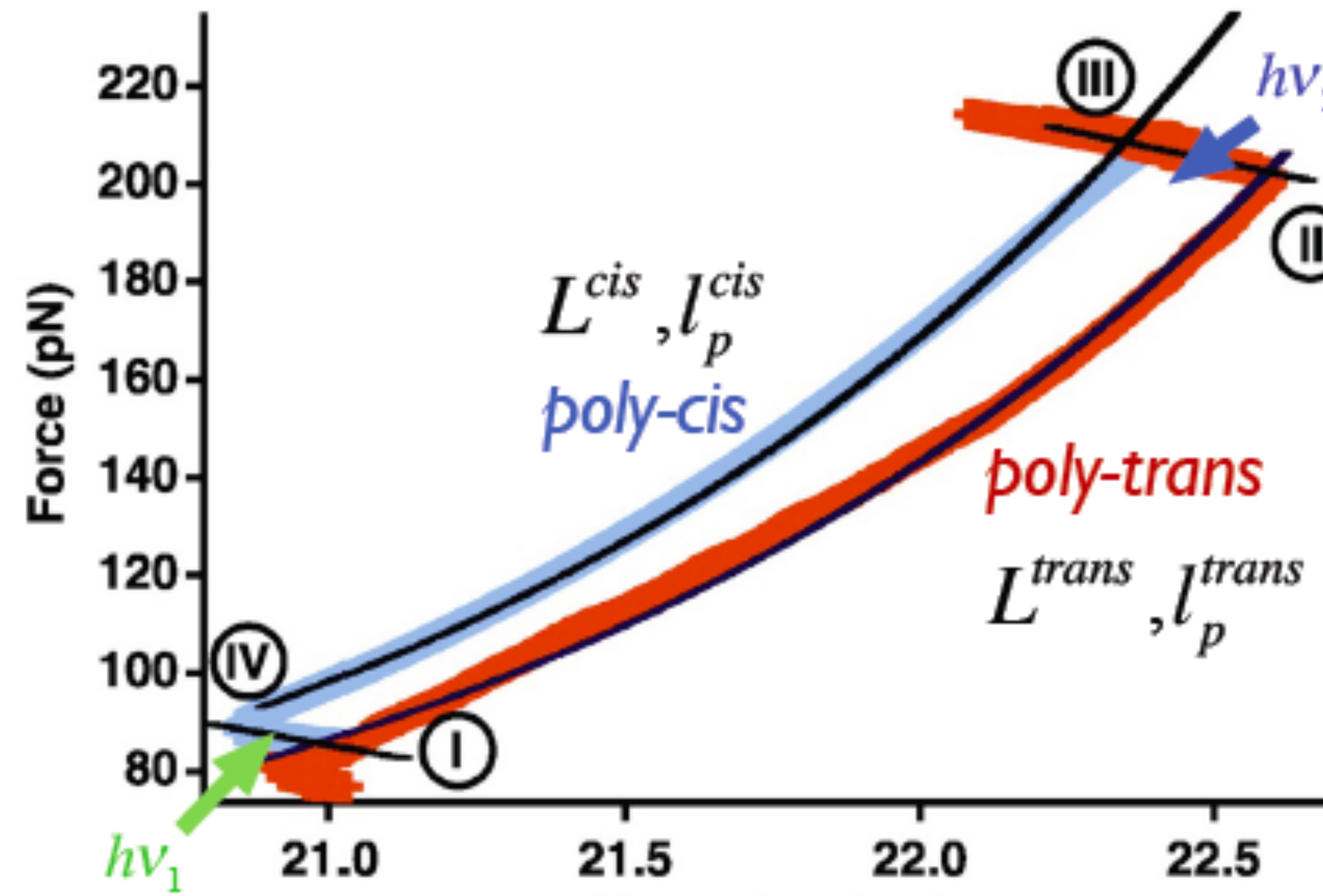


Animations from Vale and Milligan (2000) Science

ATPase activity of molecules entails "structural changes" that produce a mechanical work.

Hugel et al (2002) Science 296:1103





Kinetics of molecular motors

KINETICS OF MOTOR PROTEINS

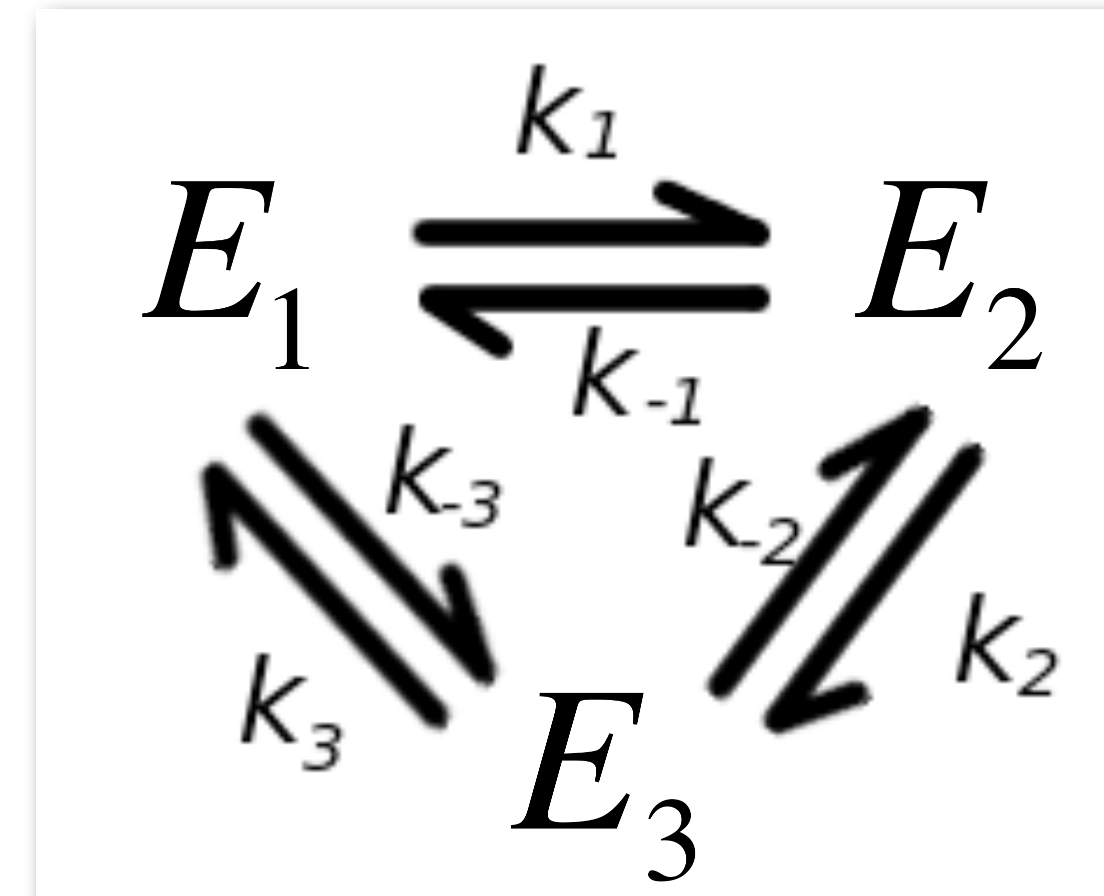
$$\frac{dP_{E_1}}{dt} = -(k_1^+ + k_3^-)P_{E_1} + k_1^- P_{E_2} + k_3^+ P_{E_3}$$

$$\frac{dP_{E_2}}{dt} = -(k_2^+ + k_1^-)P_{E_2} + k_2^- P_{E_3} + k_1^+ P_{E_1}$$

$$\frac{dP_{E_3}}{dt} = -(k_3^+ + k_2^-)P_{E_3} + k_3^- P_{E_1} + k_2^+ P_{E_2}$$

$$P_{E_1} + P_{E_2} + P_{E_3} = 1$$

Steady State Solution for Reversible Cyclic Reaction



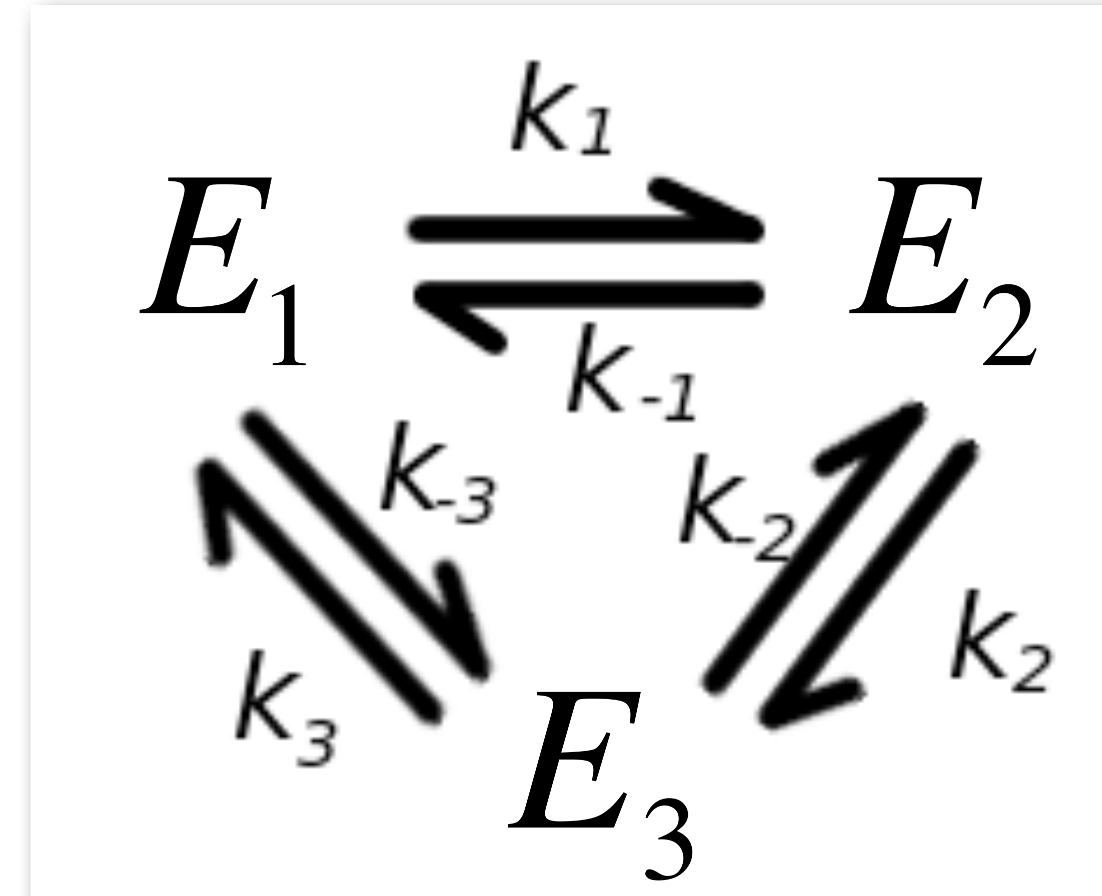
$$P_{E_1}^{ss} = \frac{k_2^+ k_3^+ + k_3^+ k_1^- + k_1^- k_2^-}{\Sigma\left(\left\{k_i^\pm\right\}\right)}$$

$$P_{E_2}^{ss} = \frac{k_3^+ k_1^+ + k_1^+ k_2^- + k_2^- k_3^-}{\Sigma\left(\left\{k_i^\pm\right\}\right)}$$

$$P_{E_3}^{ss} = \frac{k_1^+ k_2^+ + k_2^+ k_3^- + k_3^- k_1^-}{\Sigma\left(\left\{k_i^\pm\right\}\right)}$$

$$\Sigma\left(\left\{k_i^\pm\right\}\right) = k_1^+ k_2^+ + k_2^+ k_3^+ + k_3^+ k_1^+ + k_1^- k_2^- + k_2^- k_3^- + k_3^- k_1^- + k_1^- k_3^+ + k_1^+ k_2^- + k_2^+ k_3^-$$

Steady State Solution for Reversible Cyclic Reaction



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$$\Sigma\left(\left\{k_i^\pm\right\}\right) = k_1^+k_2^+ + k_2^+k_3^+ + k_3^+k_1^+ + k_1^-k_2^- + k_2^-k_3^- + k_3^-k_1^- + k_1^-k_3^+ + k_1^+k_2^- + k_2^+k_3^-$$

$$V_3 \equiv k_i^+ P_{E_i}^{ss} - k_i^- P_{E_{i+1}}^{ss}$$

j_+ **j_-**

$$= \frac{k_1^+k_2^+k_3^+ - k_1^-k_2^-k_3^-}{k_1^+k_2^+ + k_2^+k_3^+ + k_3^+k_1^+ + k_1^-k_2^- + k_2^-k_3^- + k_3^-k_1^- + k_1^-k_3^+ + k_1^+k_2^- + k_2^+k_3^-}$$

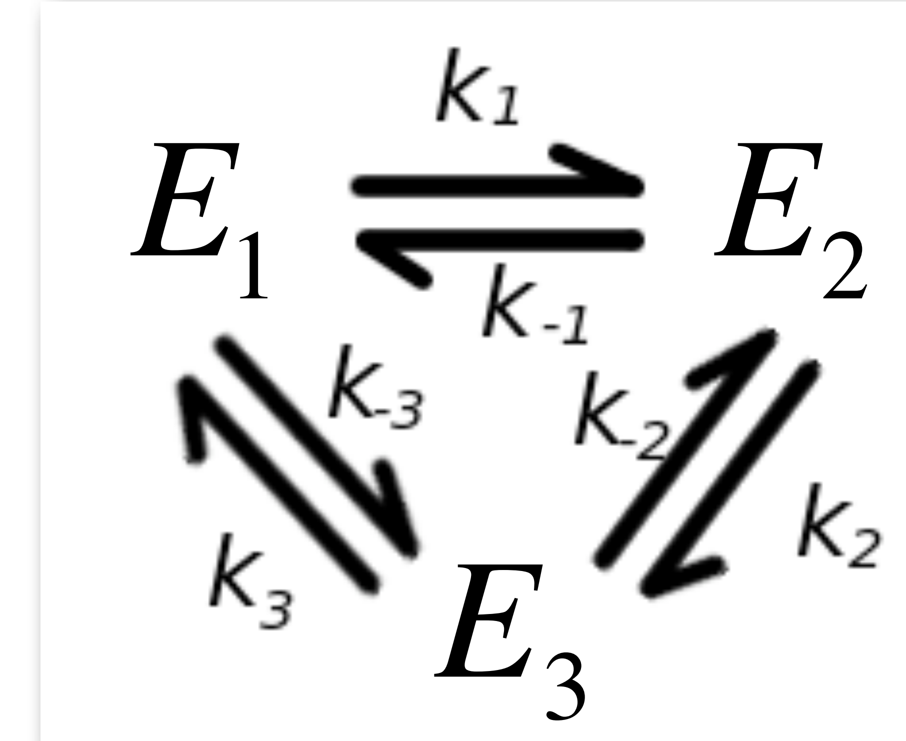
$$j(= V/\delta) = j_+ - j_-$$

$j = 0 \Rightarrow$ **Equilibrium**

$j \neq 0 \Rightarrow$ **Nonequilibrium Steady State**

$$V_3 \equiv k_i^+ P_{E_i}^{ss} - k_i^- P_{E_{i+1}}^{ss}$$

$$= \frac{k_1^+ k_2^+ k_3^+ - k_1^- k_2^- k_3^-}{k_1^+ k_2^+ + k_2^+ k_3^+ + k_3^+ k_1^+ + k_1^- k_2^- + k_2^- k_3^- + k_3^- k_1^- + k_1^- k_3^+ + k_1^+ k_2^- + k_2^+ k_3^-}$$

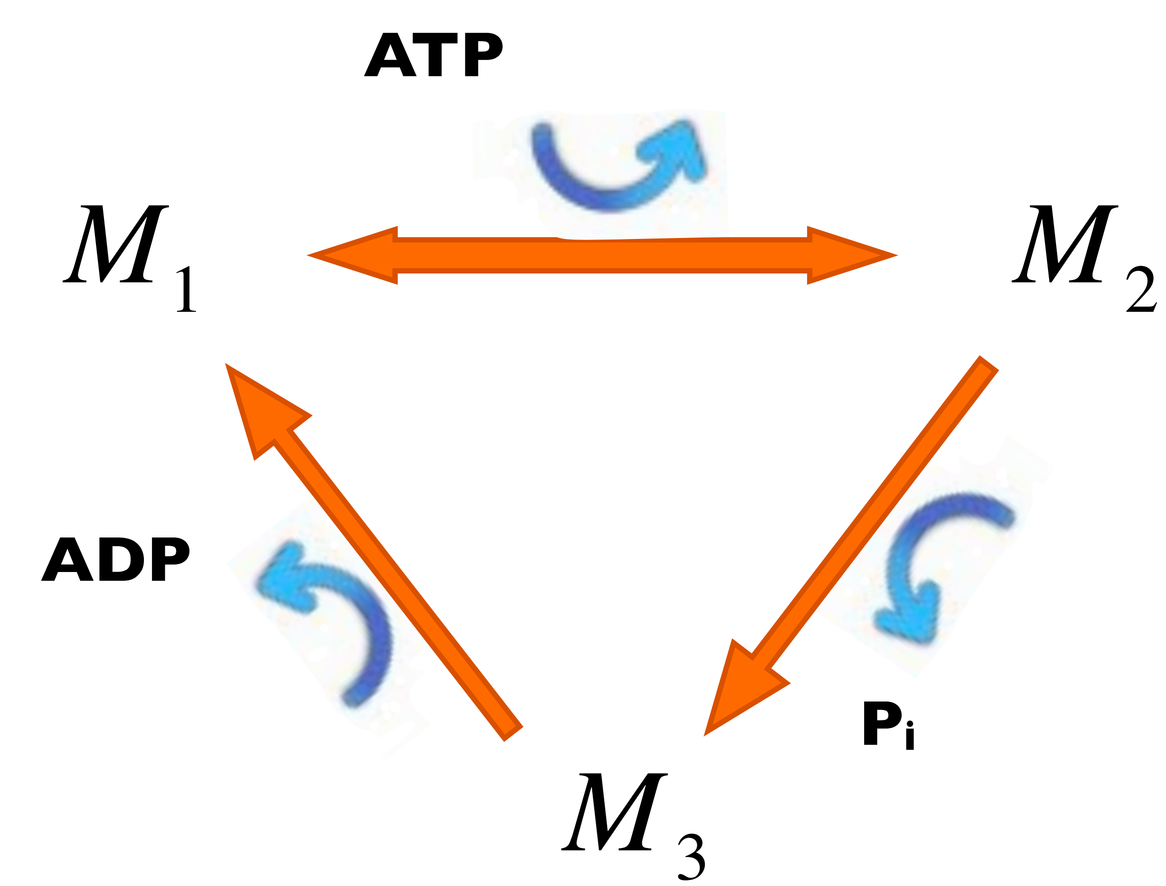


$$k_1^+ \to k_1^+[T]$$

$$k_2^- \to k_2^-[P]$$

$$k_3^- \to k_3^-[D]$$

$$V = \delta \frac{\prod_{i=1}^{3} k_i^+[T] - \prod_{i=1}^{3} k_i^-[D][P]}{\sum\left(\left\{k_i^\pm\right\}\right)}$$

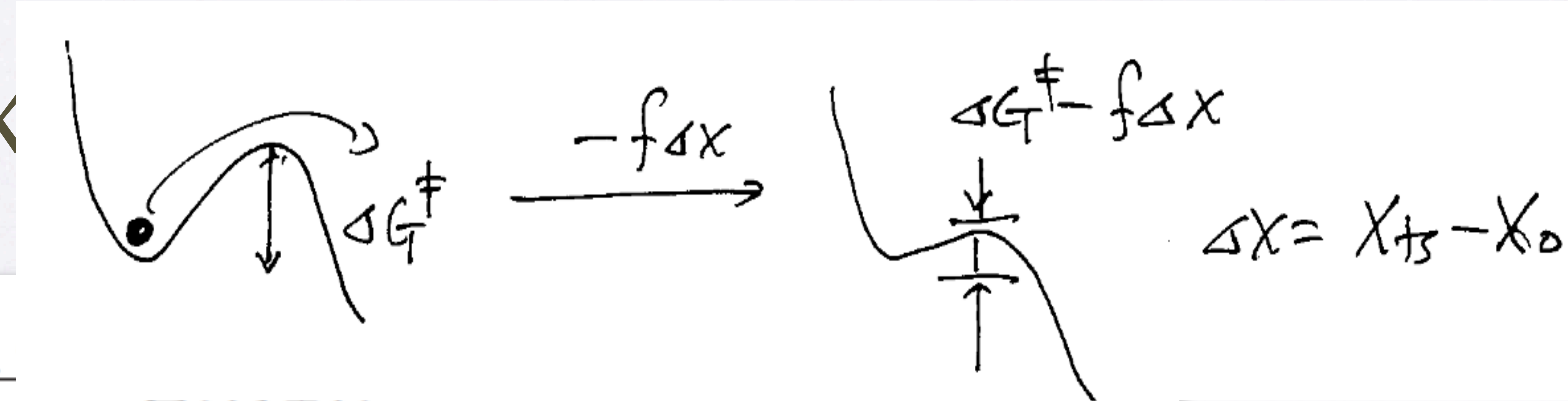


$$K = \frac{j_+}{j_-} = \frac{k_1^+ k_2^+ k_3^+ [T]}{k_1^- k_2^- k_3^- [D][P]}$$

$$1 = \frac{k_1^+ k_2^+ k_3^+ [T]_{eq}}{k_1^- k_2^- k_3^- [D]_{eq}[P]_{eq}}$$

$$\Delta\mu = -k_B T \log K = k_B T \log\left(\left\{\frac{[D][P]}{[T]}\right\} \Big/ \left\{\frac{[D]_{eq}[P]_{eq}}{[T]_{eq}}\right\}\right)$$

Under ex



$$V = \delta \frac{\prod_{i=1}^{N}}{\Sigma(\{k_i^{\pm}\})}$$

$$= \delta \frac{k_T^+[T]k_S^+k_{hyd}^+k_{-P}^+k_{-D}^+ - k_T^-k_S^-k_{hyd}^-k_{-P}^-[P]k_{-D}^-[D]}{\Sigma(\{k_i^{\pm}\})}$$

$$k_i^{\pm} \to k_i^{\pm} \exp\left(\pm f\delta_i^{\pm}/k_BT\right)$$

j₊   *j₋*

$$V(f) = \delta \frac{\left(\prod_{i=1}^{N} k_i^+[T]\right) e^{-f\sum_i \delta_i^+/k_BT} - \left(\prod_{i=1}^{N} k_i^-[D][P]\right) e^{f\sum_i \delta_i^-/k_BT}}{\Sigma(f)}$$

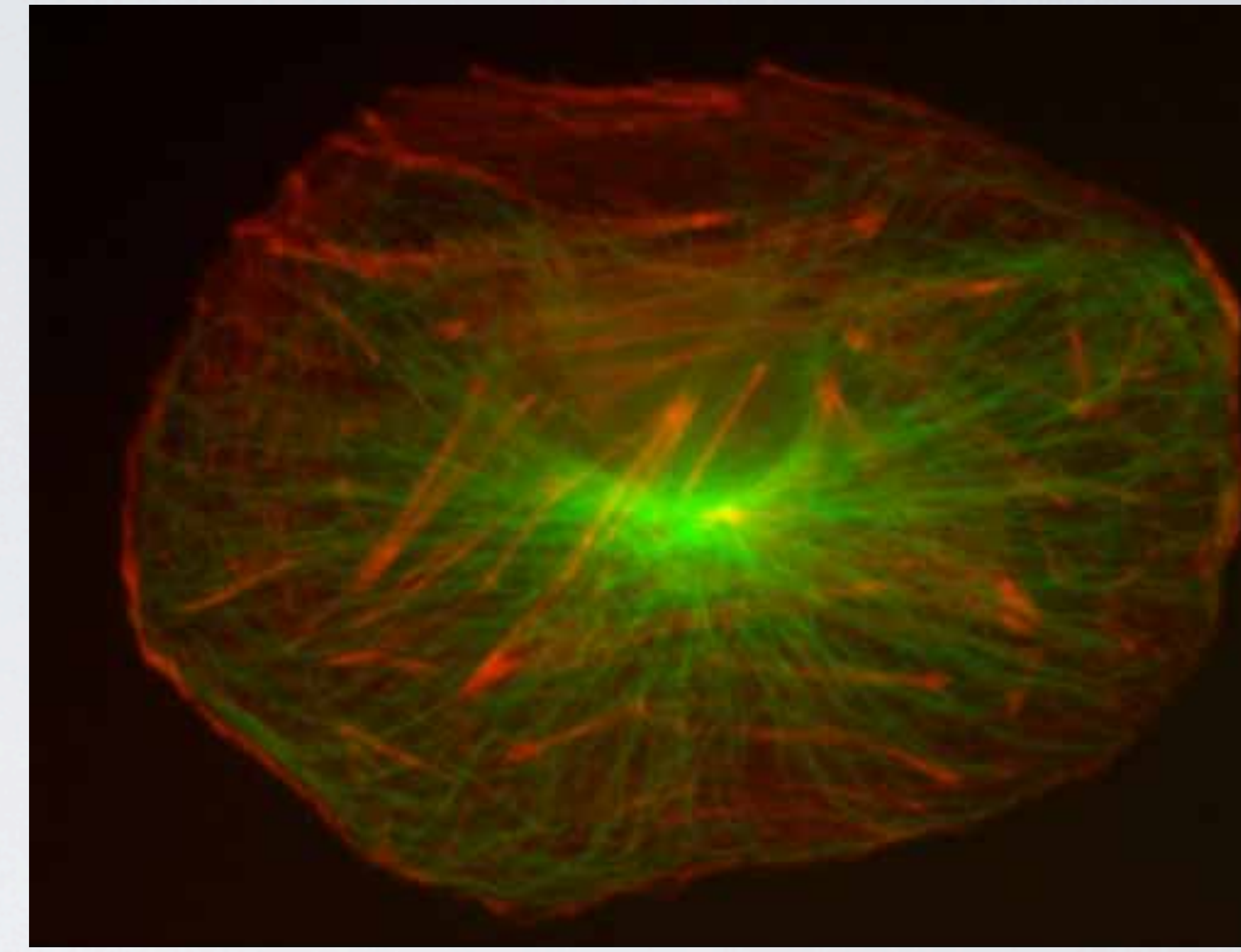
$$\sum_i \delta_i^+ + \sum_i \delta_i^- = \delta = 8 \text{ nm}$$

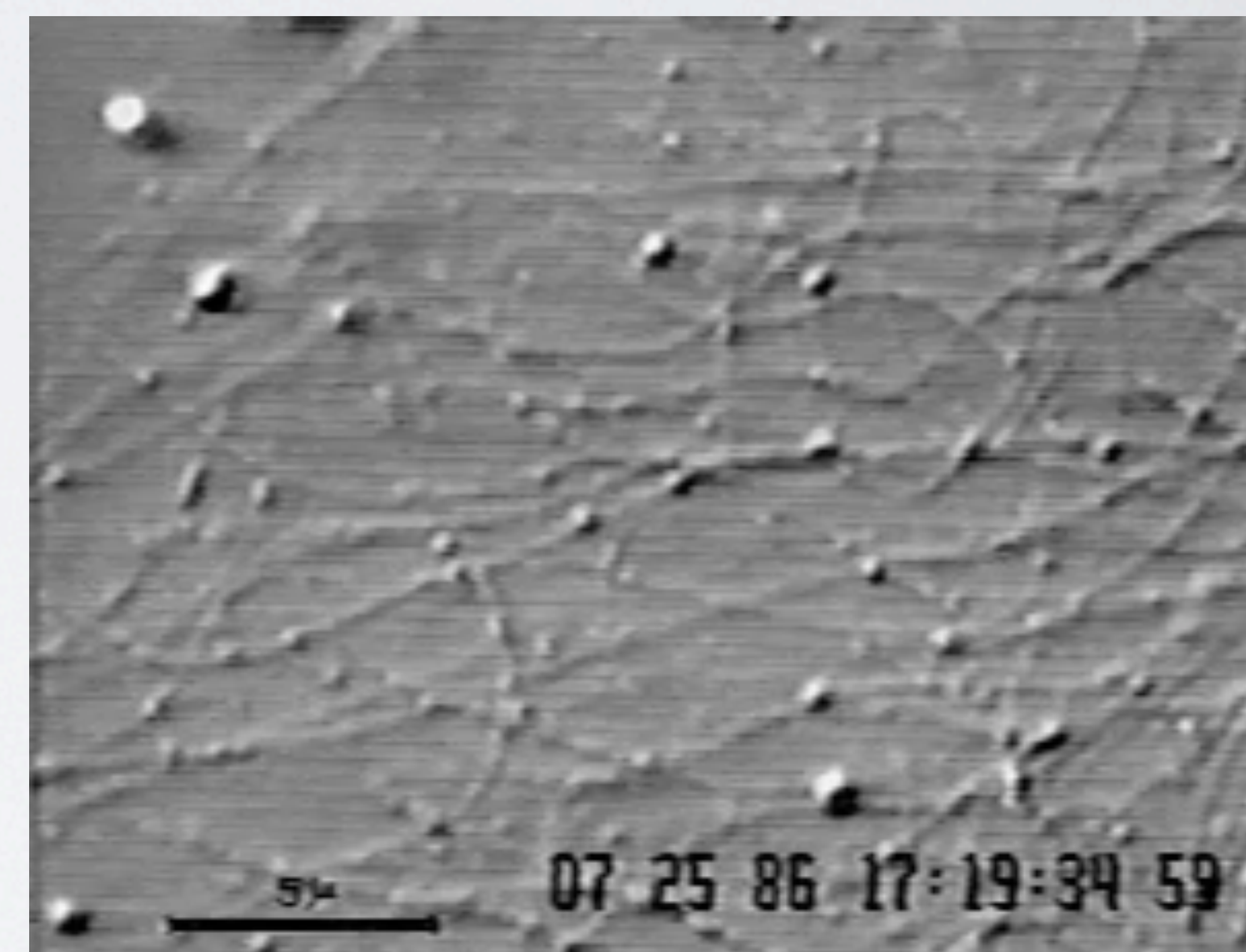
$$K = j_+/j_- = K^o e^{-f\delta/k_BT} = \left(K_{eq}\frac{[T]}{[D][P]}\right) e^{-f\delta/k_BT}$$

Kinesins

Transport motors in cellular systems

- Transport motors (kinesin, myosin, dynesin) carry cellular material along the complex network of cytoskeletal filaments such as microtubules and actin.
- Molecular motors catalyze the hydrolysis of ATP so as to proceed along cytoskeletal filament.
- Kinesin's speed (Rapid transport of organelle along the axon is accomplished by directed motions of kinesin)
  - V ~ 1μm/sec ➡ To travel 1 m ➡ t ~ 11 days.
  - cf. D ~ 10 μm^2/sec ➡ To travel 1m ➡ t > 300 years

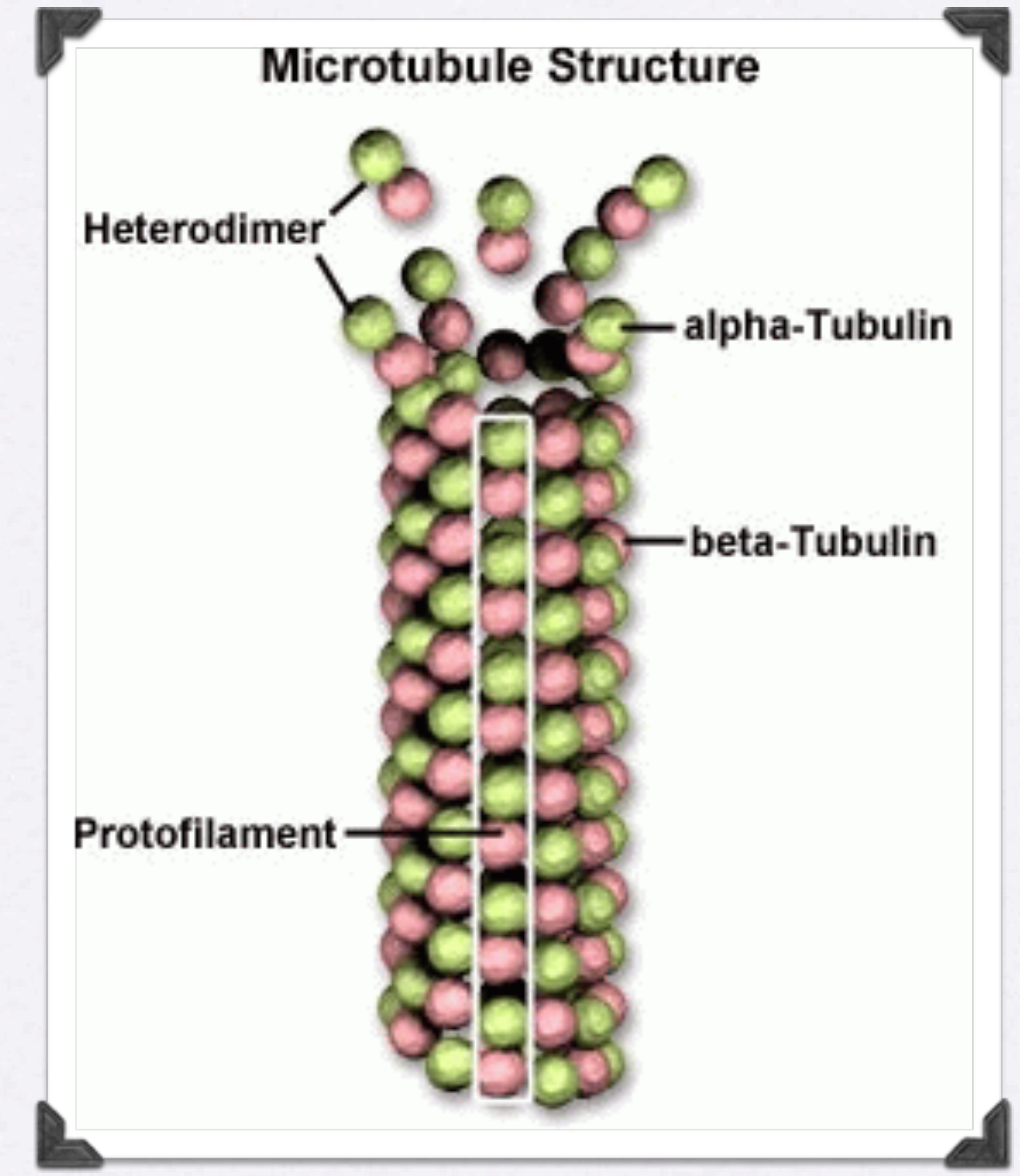


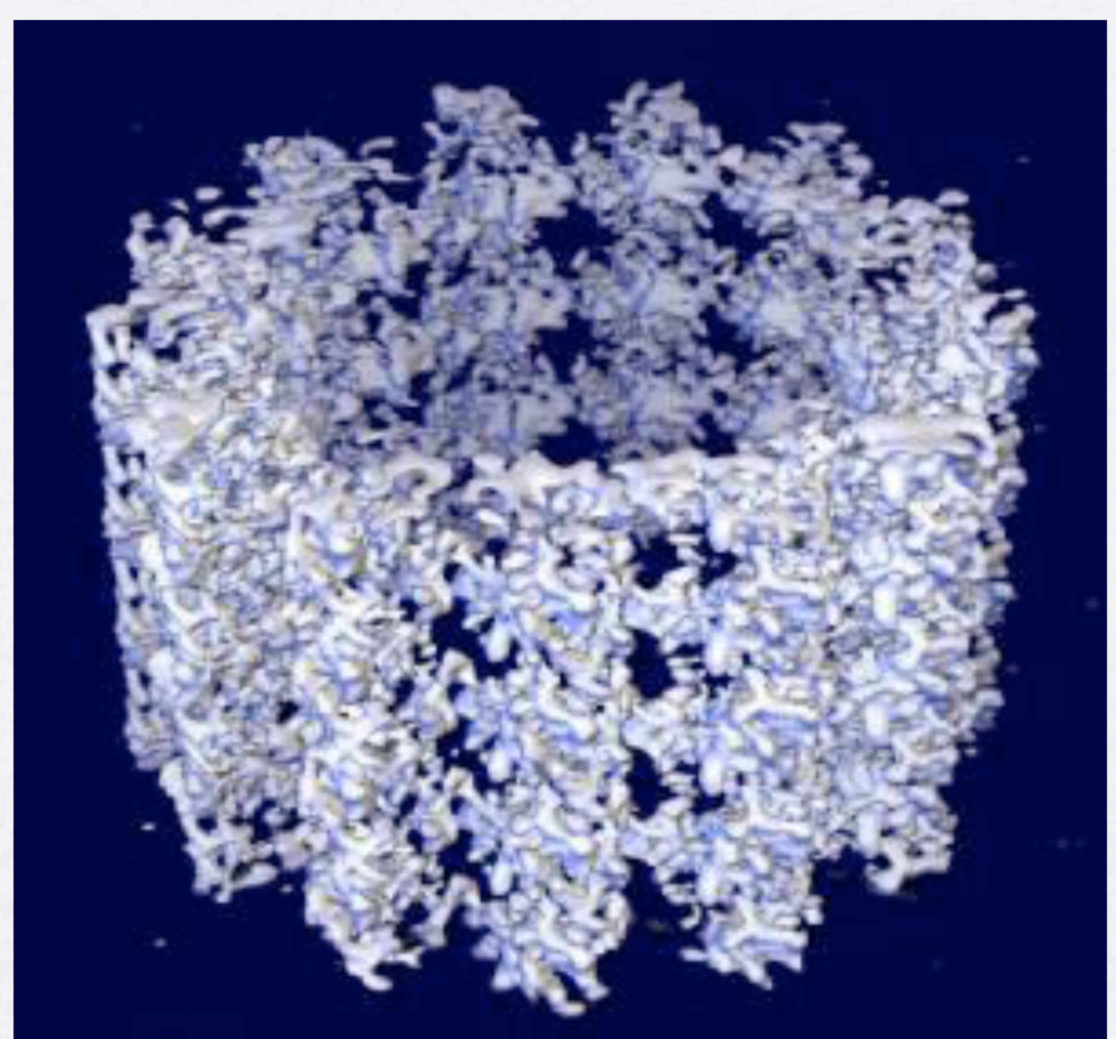


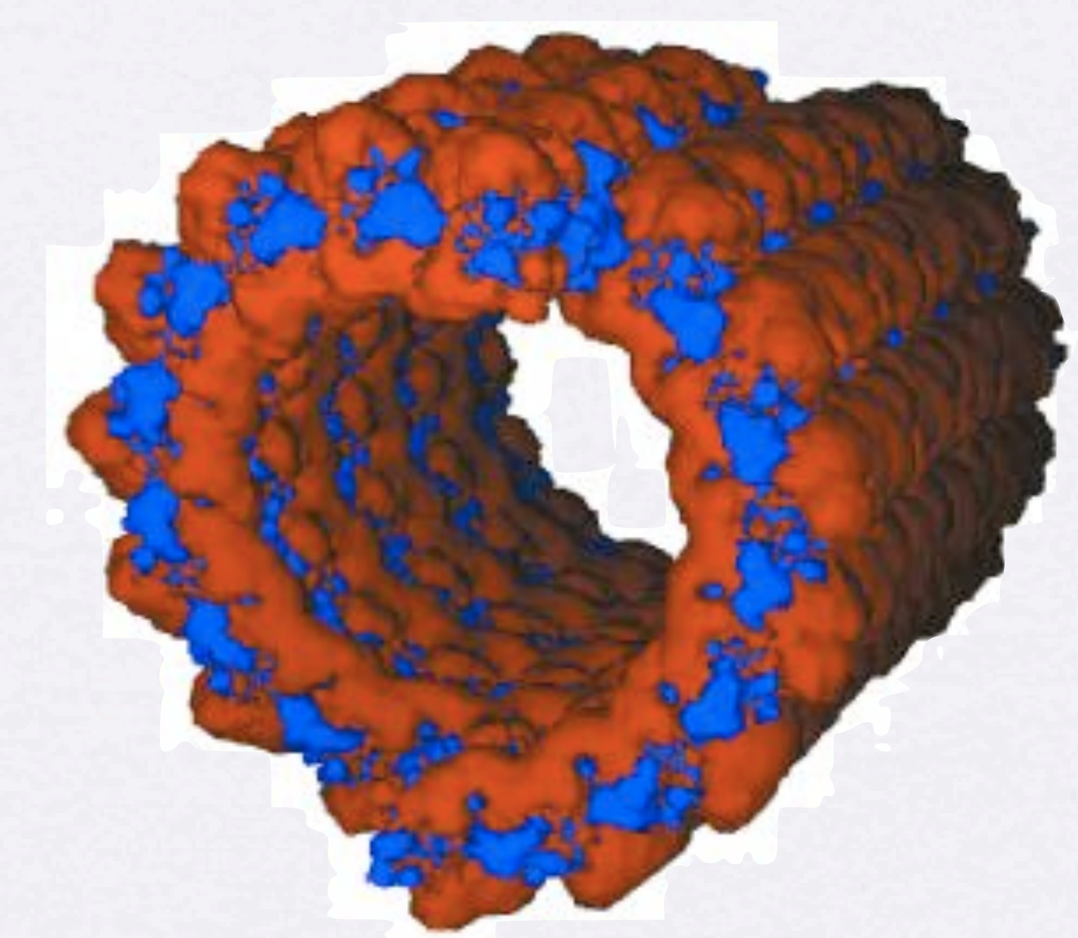
axon of giant squid

Microtubules

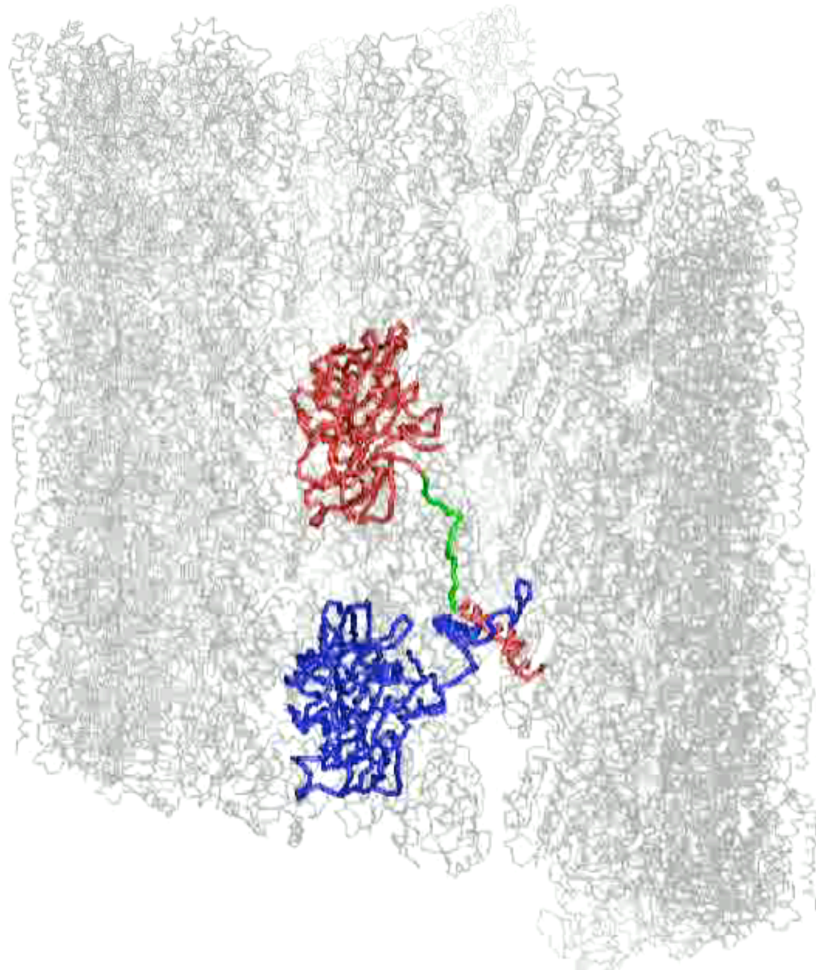
- consist of multiple alpha and beta-tubulin dimers.
- In vivo : 13 protofilament, straight along the axis
- In vitro : 11-15 protofilament, helical along the axis.
- Each dimer is highly charged (net charge = -34 e)



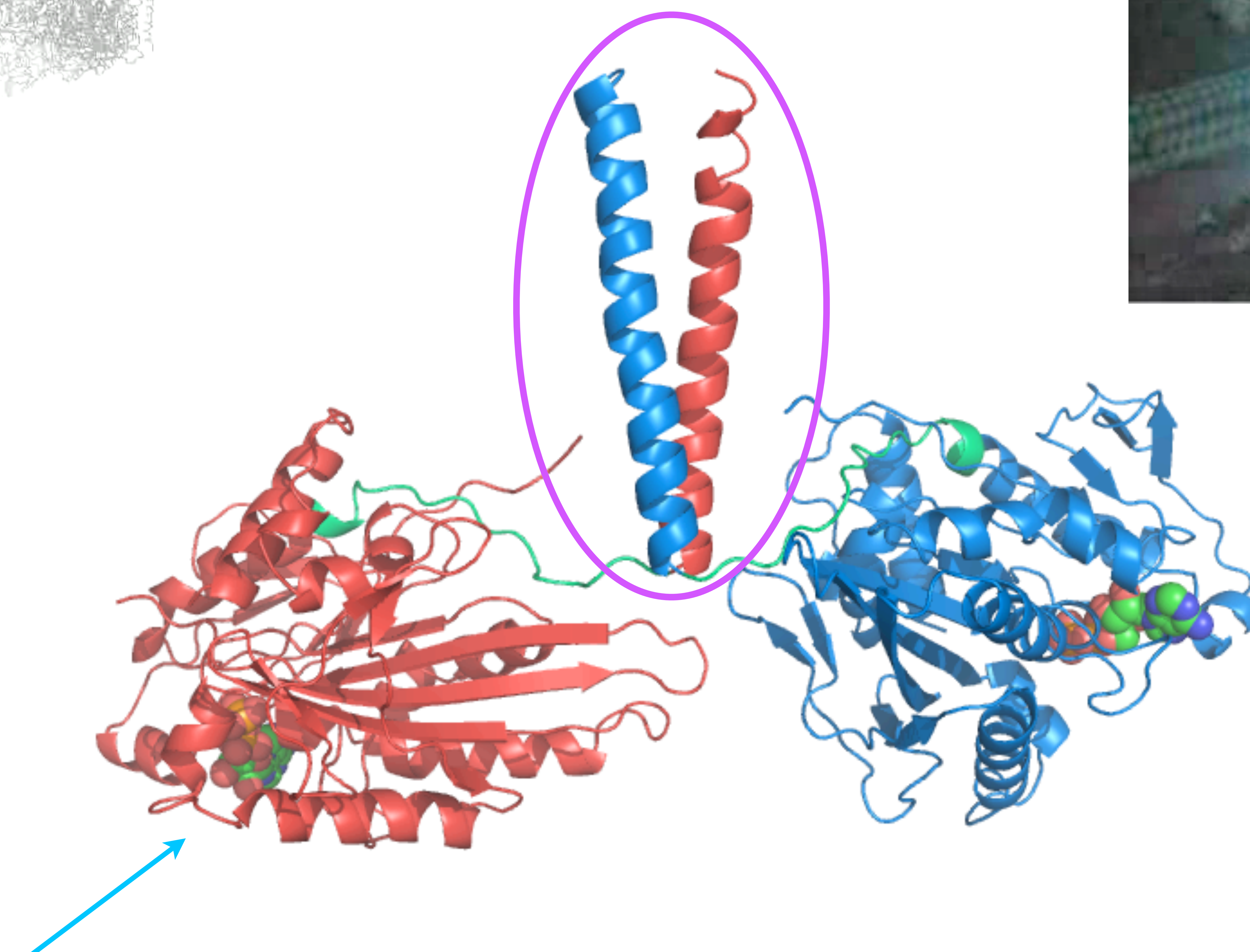




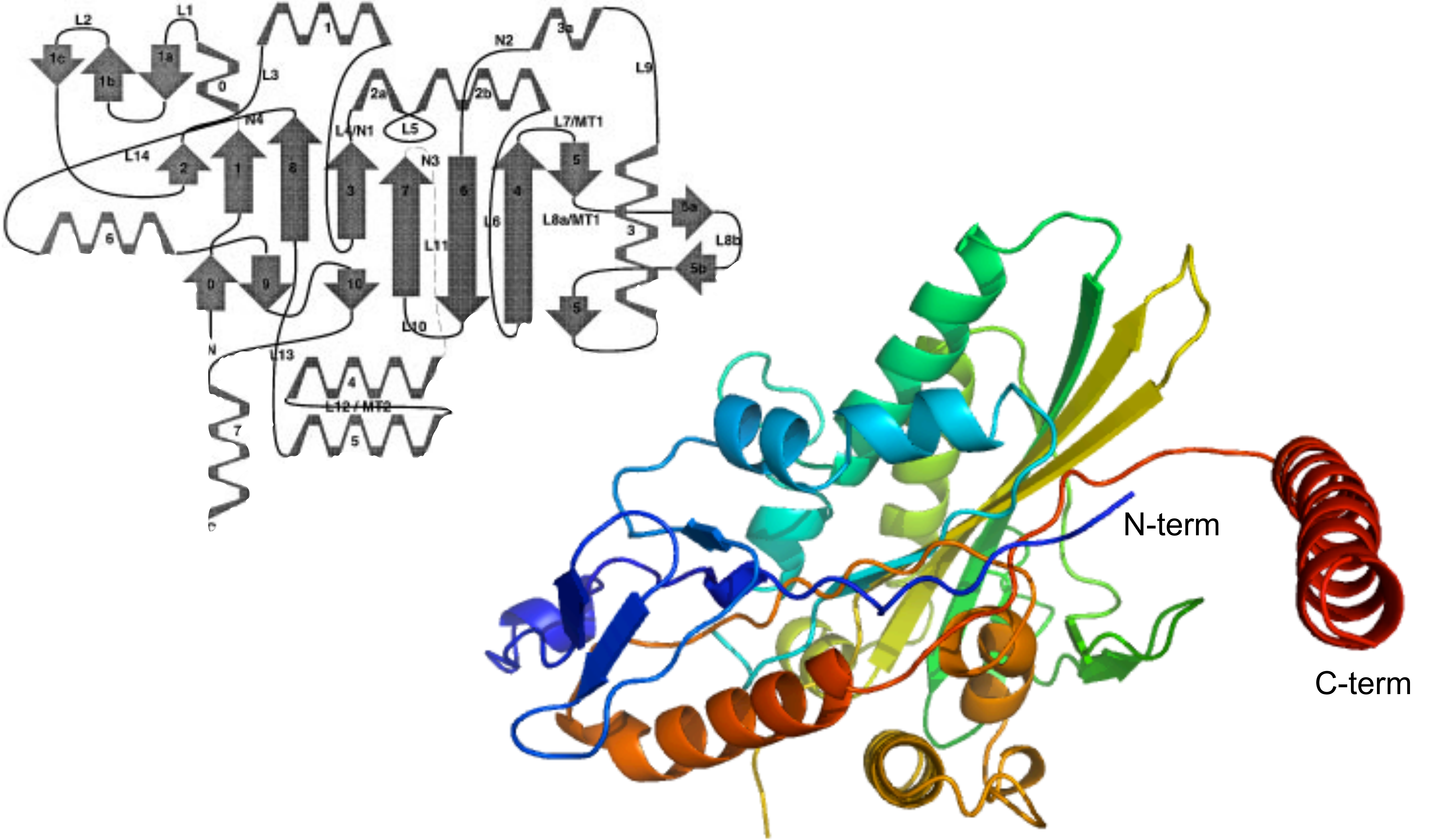
Crystal structure of kinesin-1

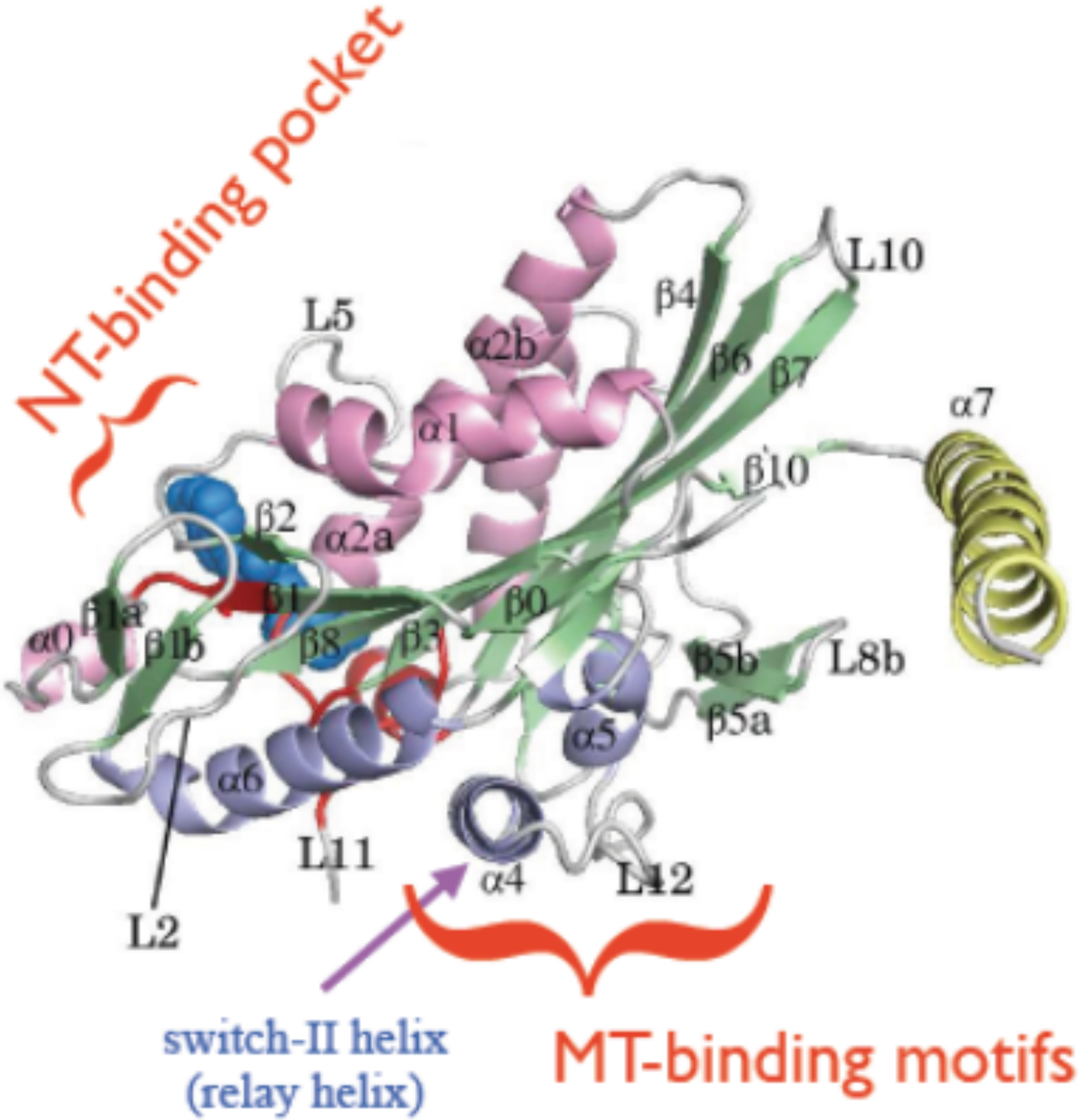


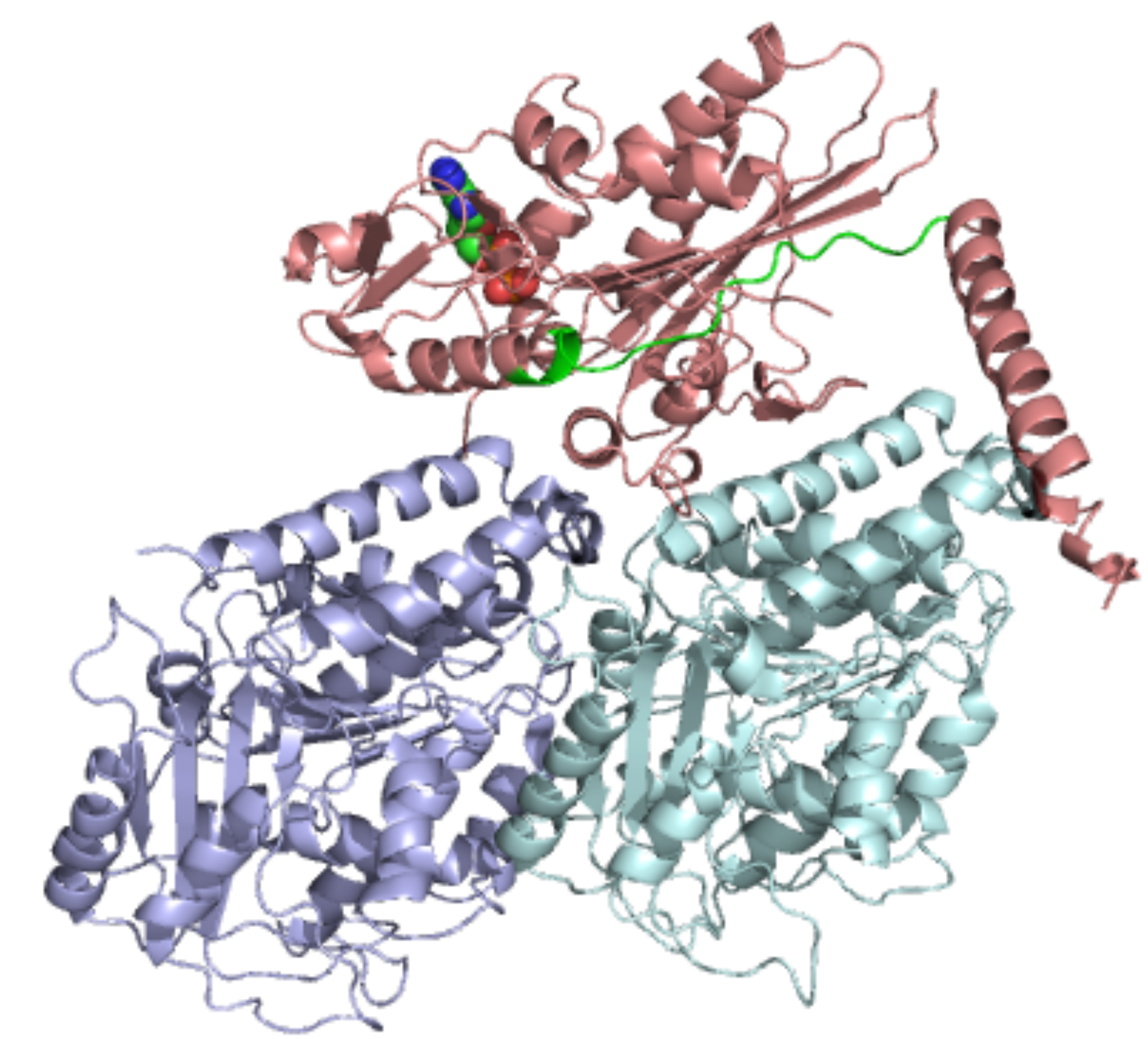
coiled-coil association

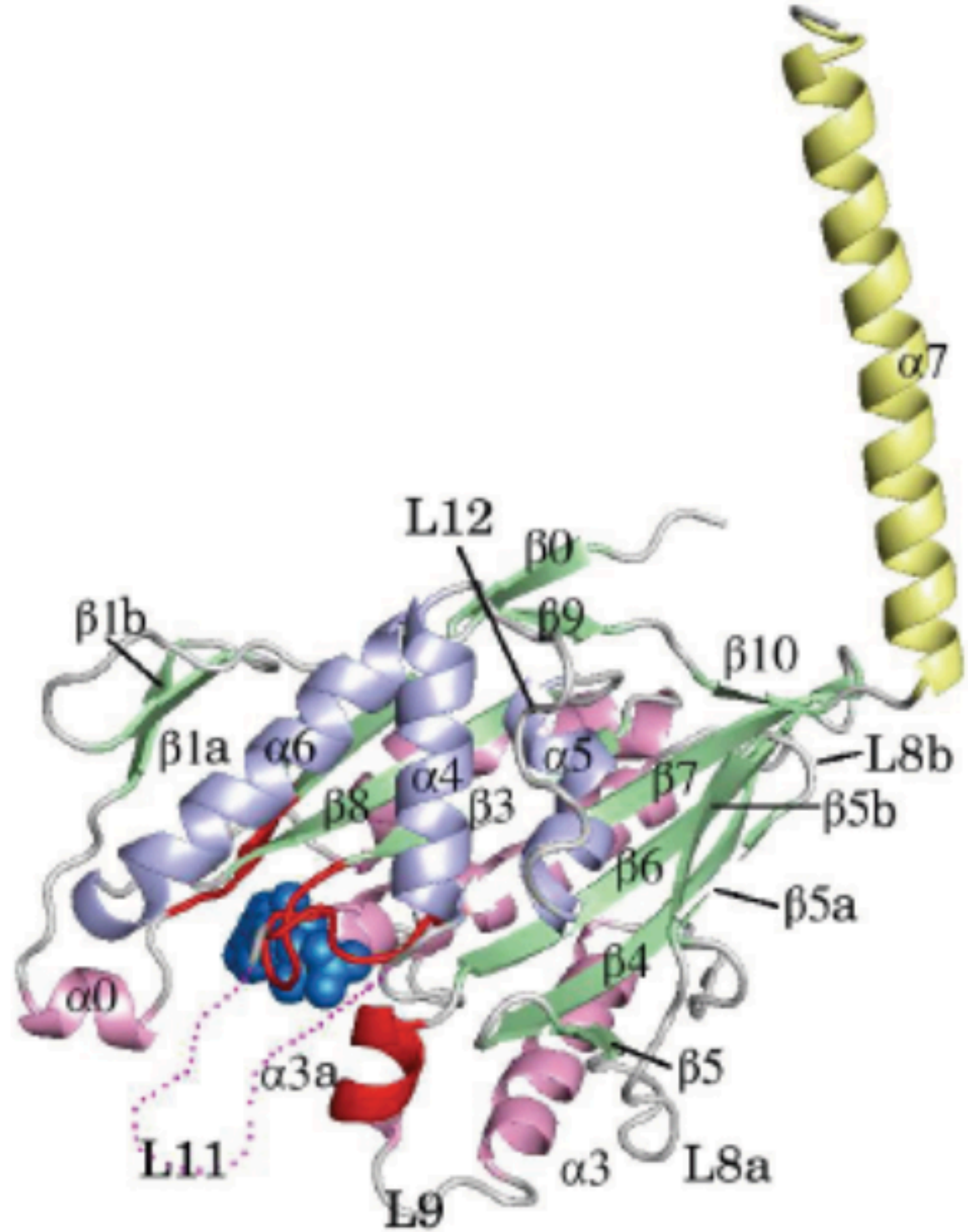


nucleotide (ATP, ADP.Pi, ADP) binding site

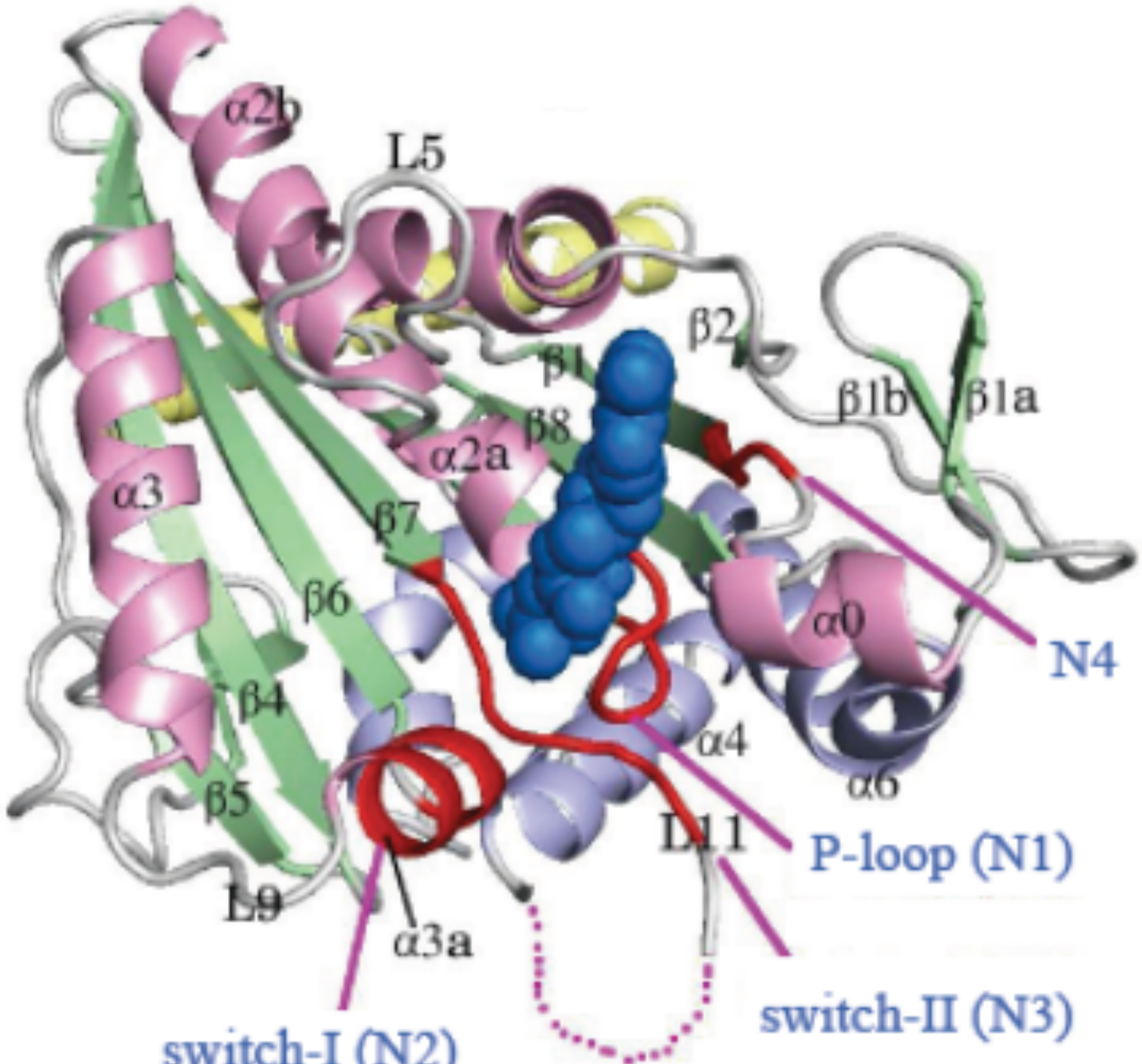

L1
L2
L3
L4/N1
L5
N2
N3
N4
L6
L7/MT1
L8a/MT1
L8b
L9
L10
L11
L12 / MT2
L13
L14
N
N-term
C-term


NT-binding pocket
L5
α2b
L10
β4
β6
β7
α1
α7
β10
β2
α2a
β1a
β1
β0
α0
β1b
β8
β3
β5b
L8b
β5a
α5
α6
L11
α4
L12
L2
switch-II helix
(relay helix)
MT-binding motifs

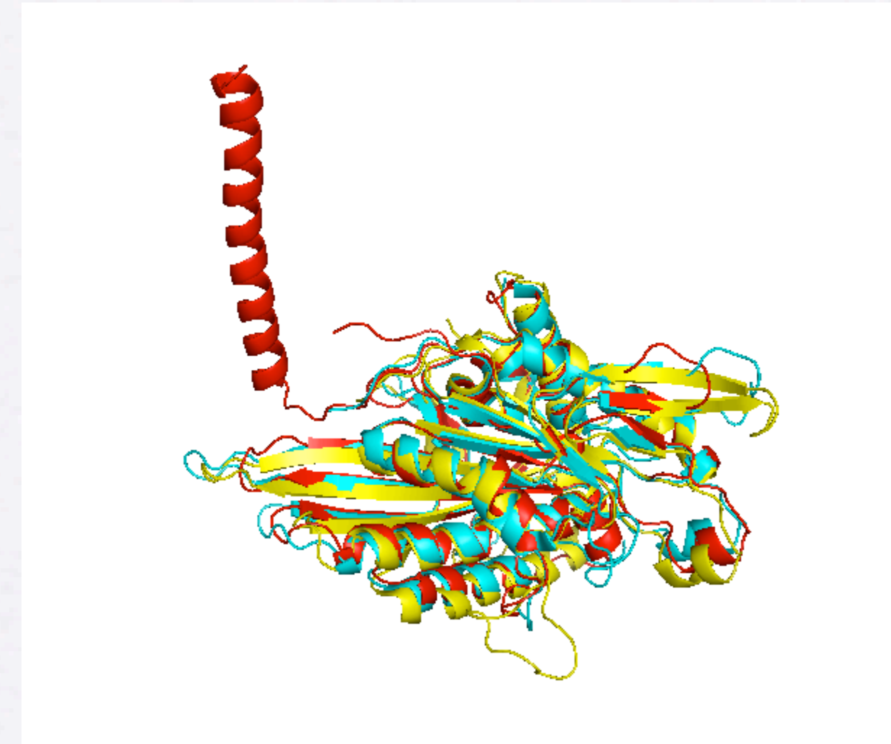




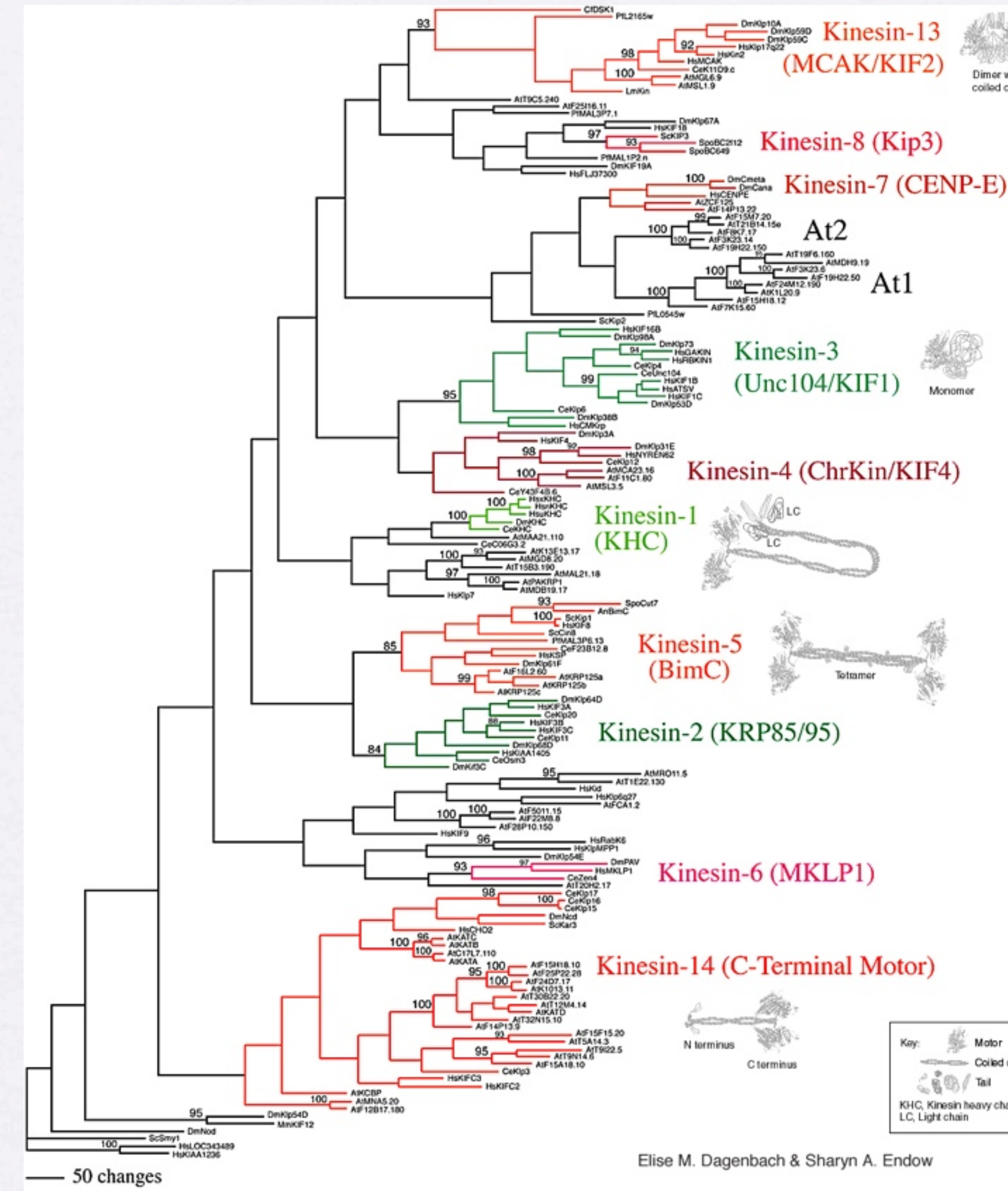
α7
L12
β0
β9
β1b
β10
β1a
α6
α5
L8b
α4
β7
β8
β3
β5b
β6
β5a
β4
α0
β5
α3a
L11
L8a
α3
L9


α2b
L5
β2
β1
β1b
β1a
β8
α2a
α3
β7
β6
α0
N4
β4
α4
α6
β5
L11
P-loop (N1)
L9
α3a
switch-II (N3)
switch-I (N2)

Kinesin family



- 45 kinesin genes
- kinesin-1 : Conventional kinesin, responsible for material transport. (the most well studied kinesin)
- kinesin-5 (Eg5) : kinesin tetramer responsible for bipolar spindle formation leading to the cell division.
- Single-headed kinesin (KIF1A)
- kinesin-13 : Depolymerization of microtubules
- (-) end-directed kinesin (NCD).



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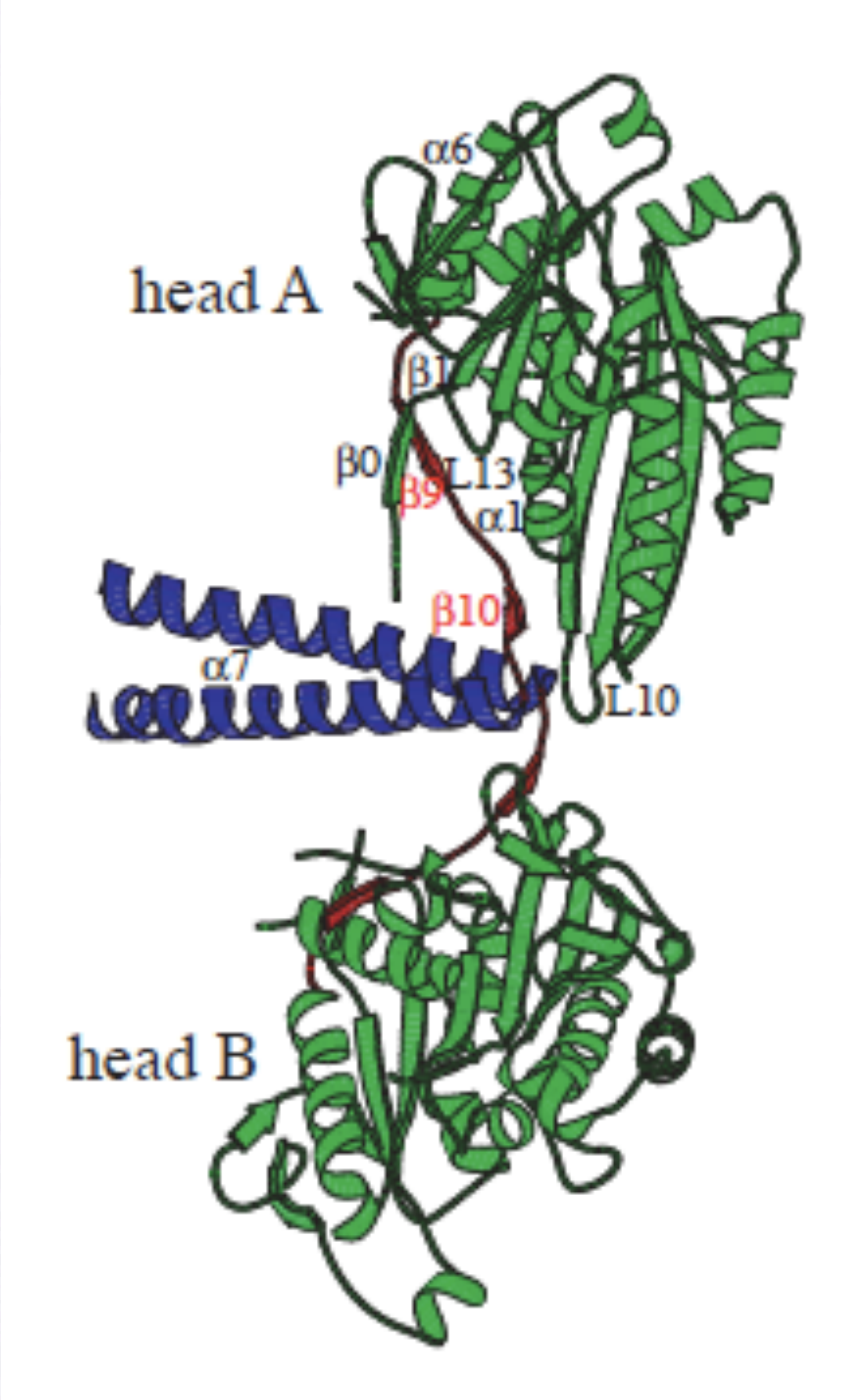

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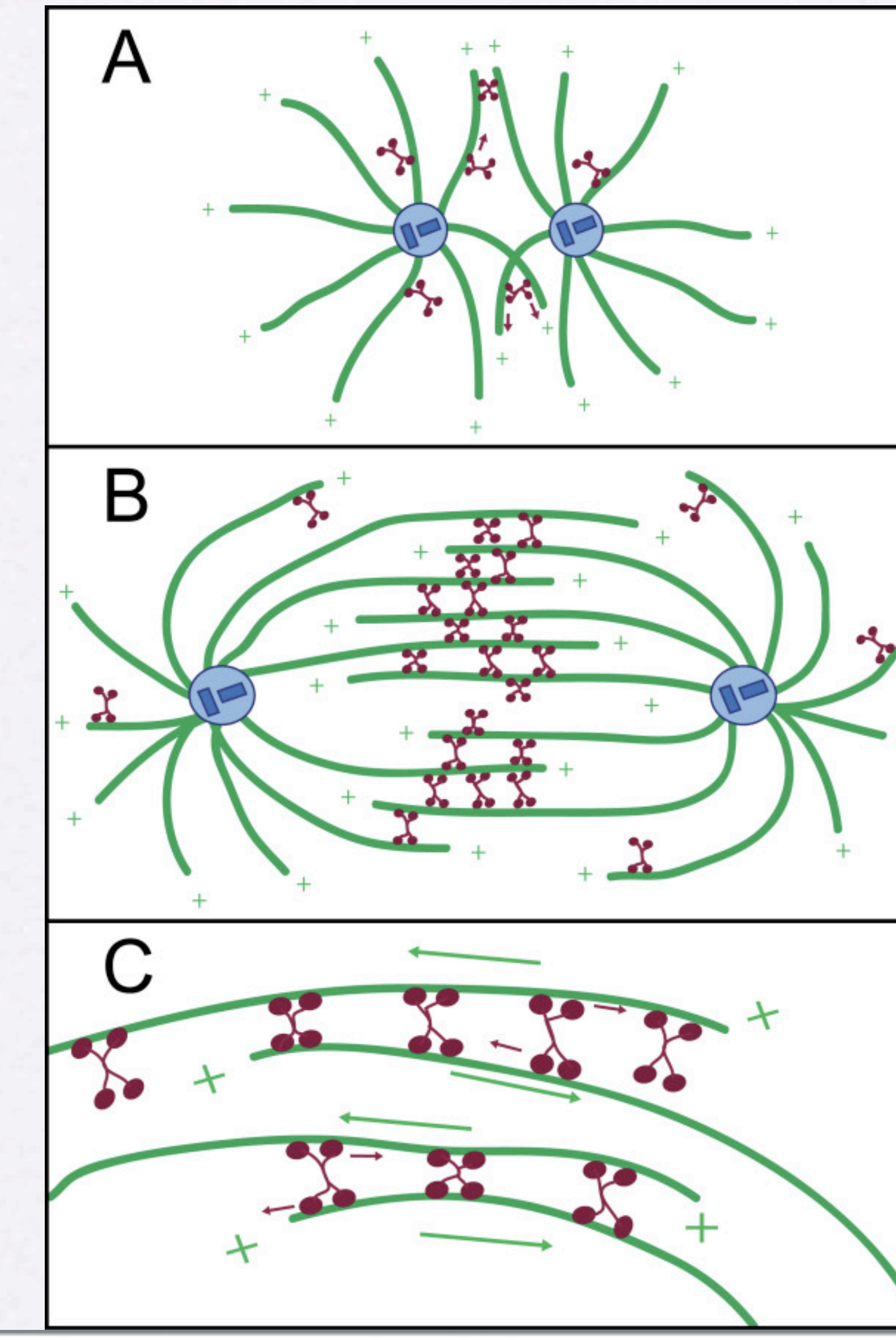

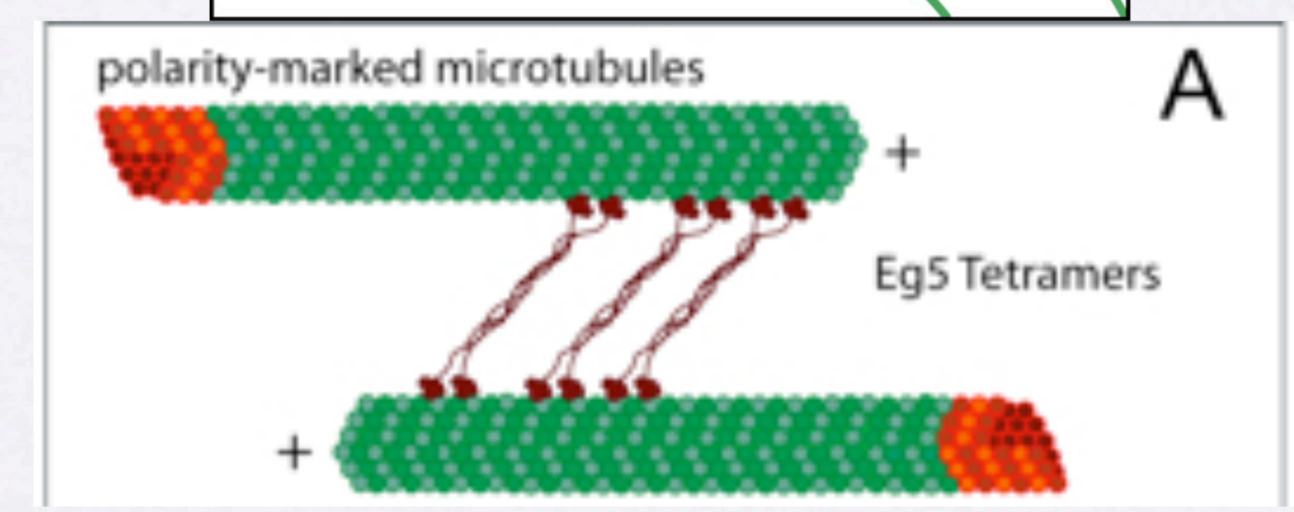

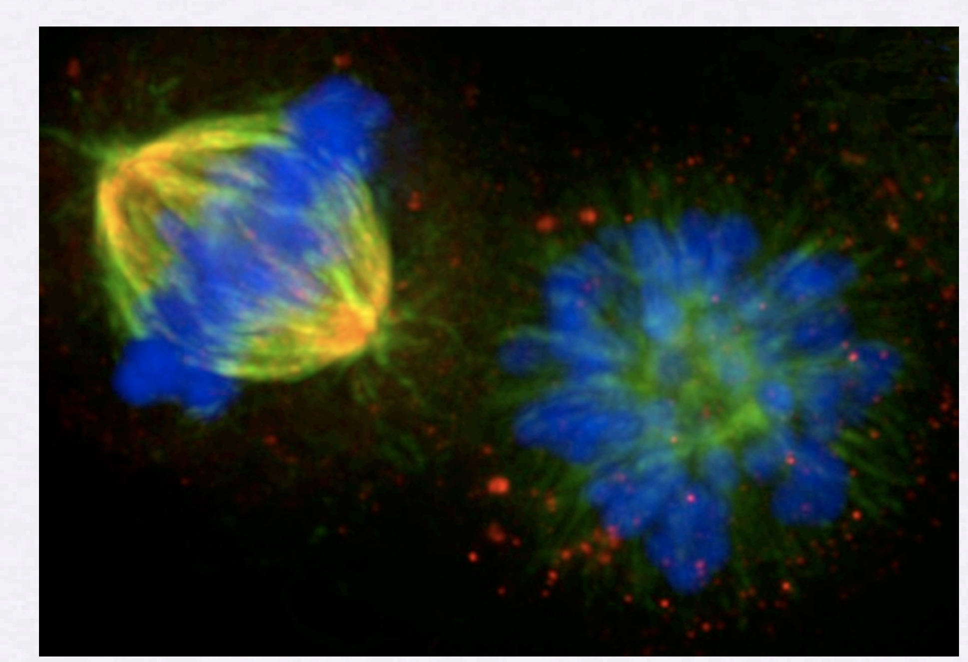

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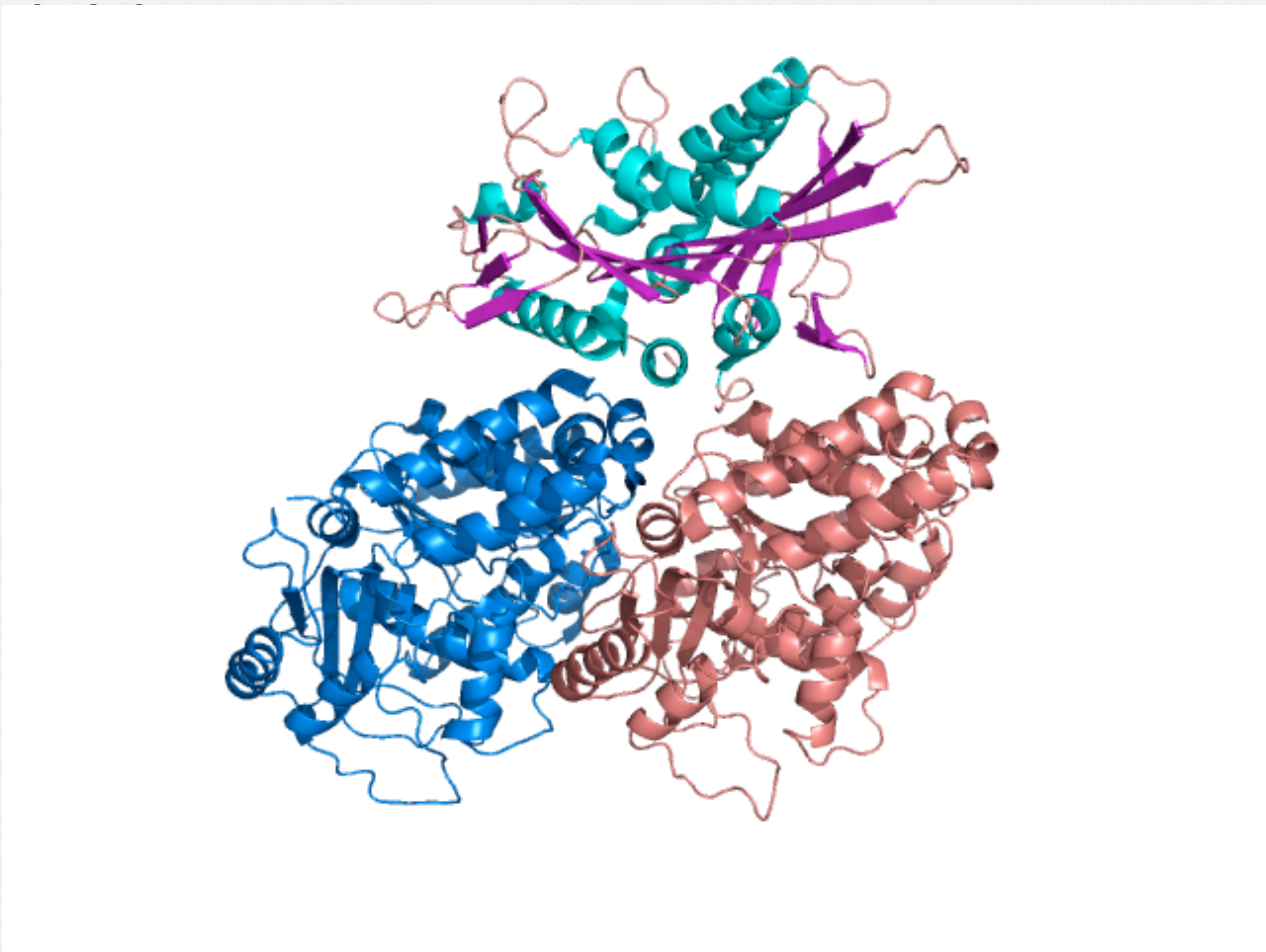
- Single-headed kinesin (KIF1A)
- kinesin-13 : Depolymerization of microtubules
- (-) end-directed kinesin (NCD).



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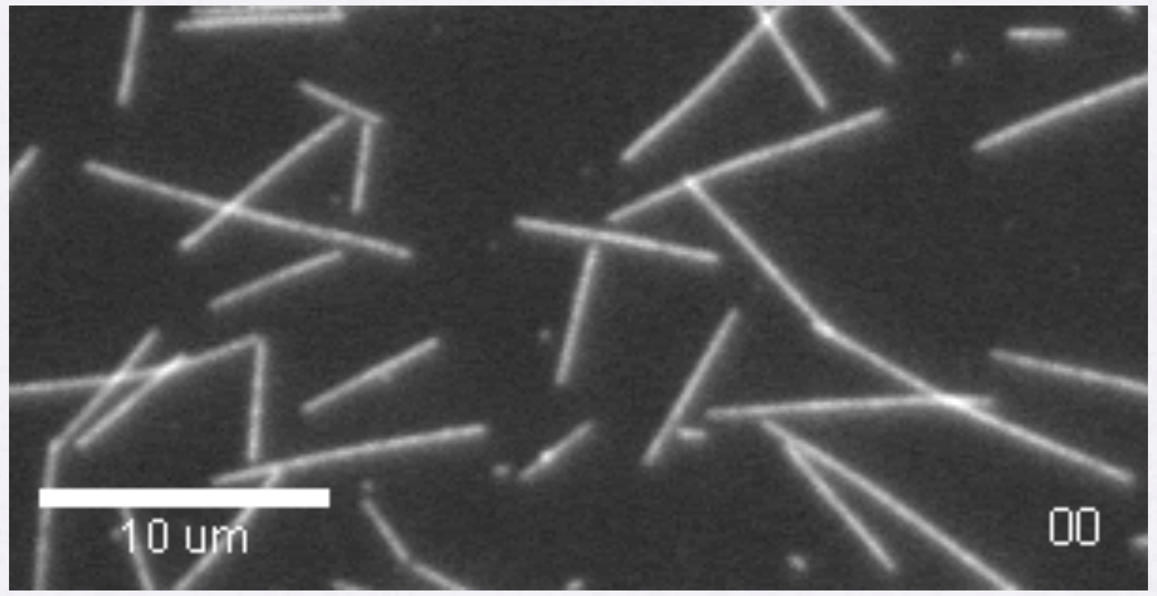

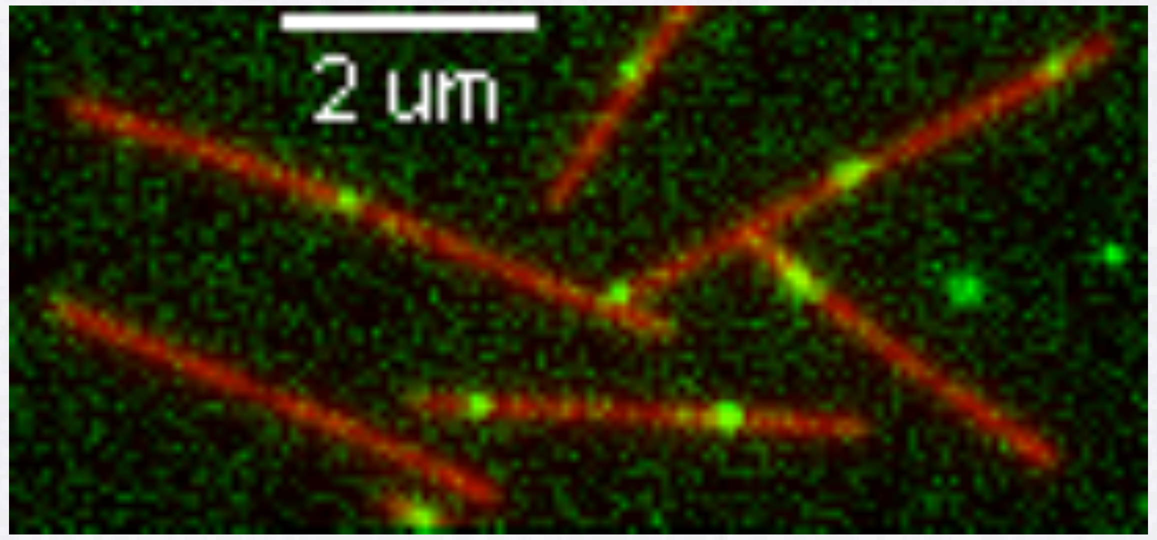

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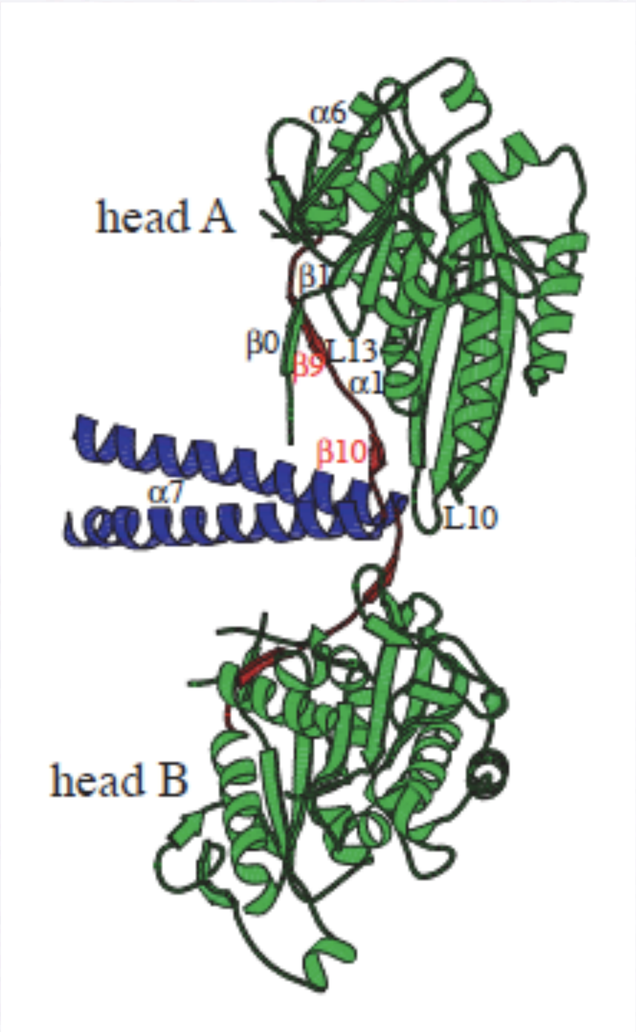
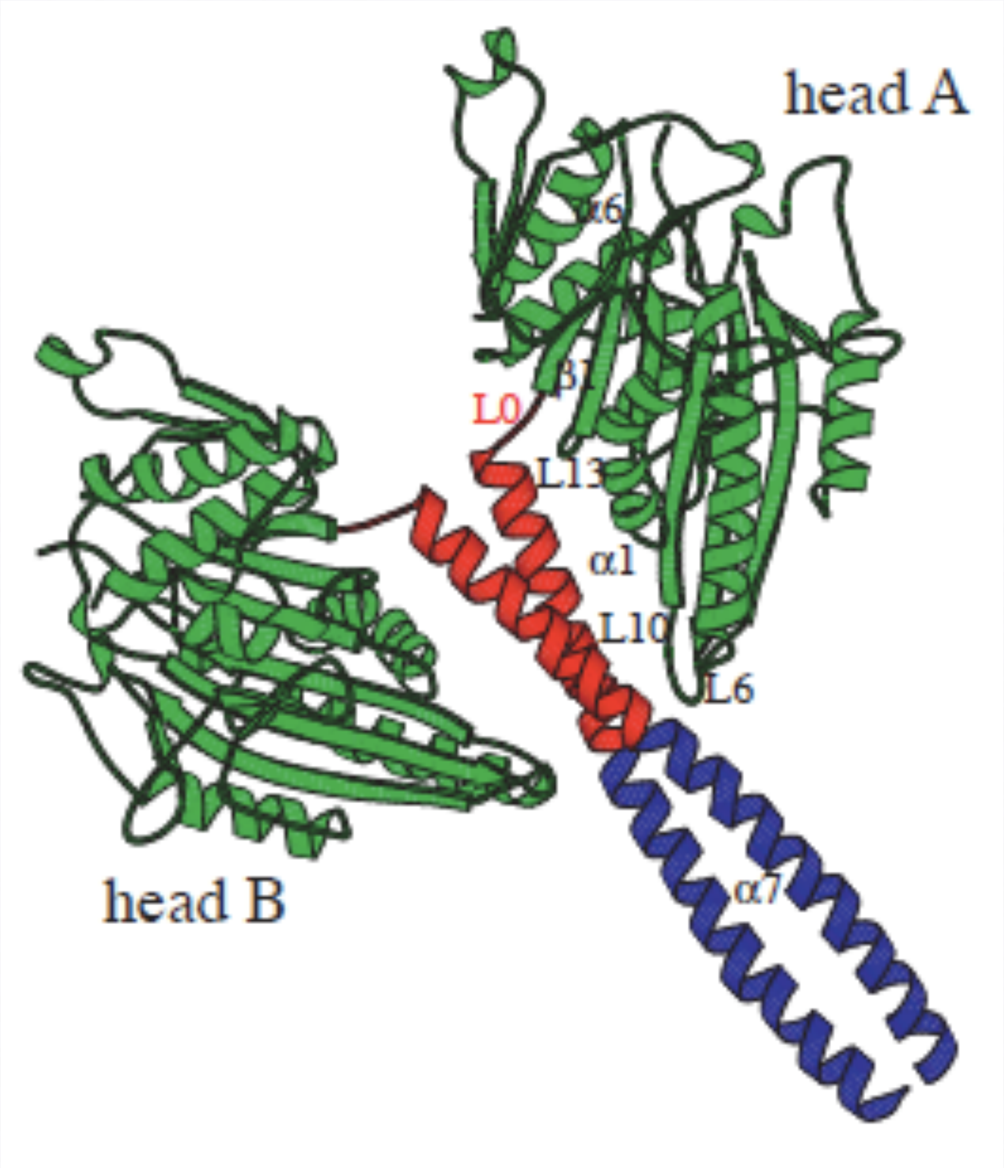
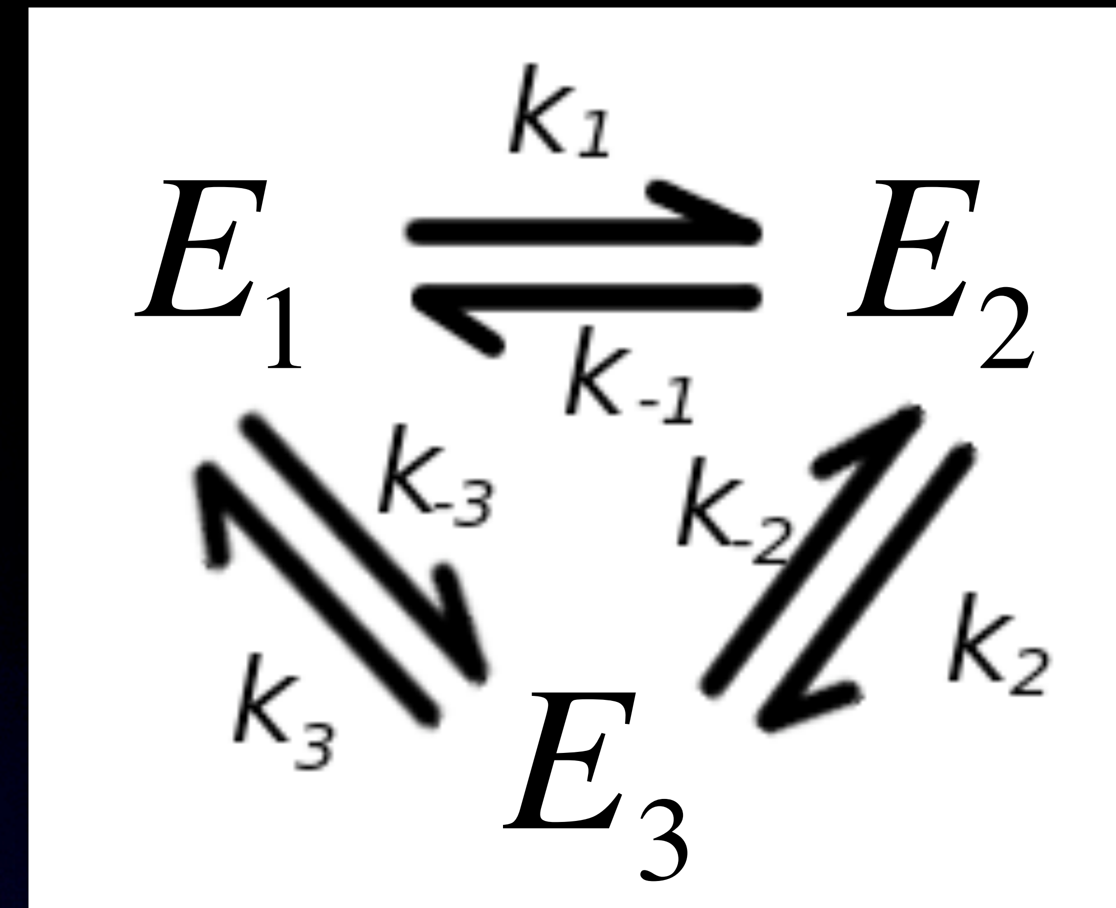


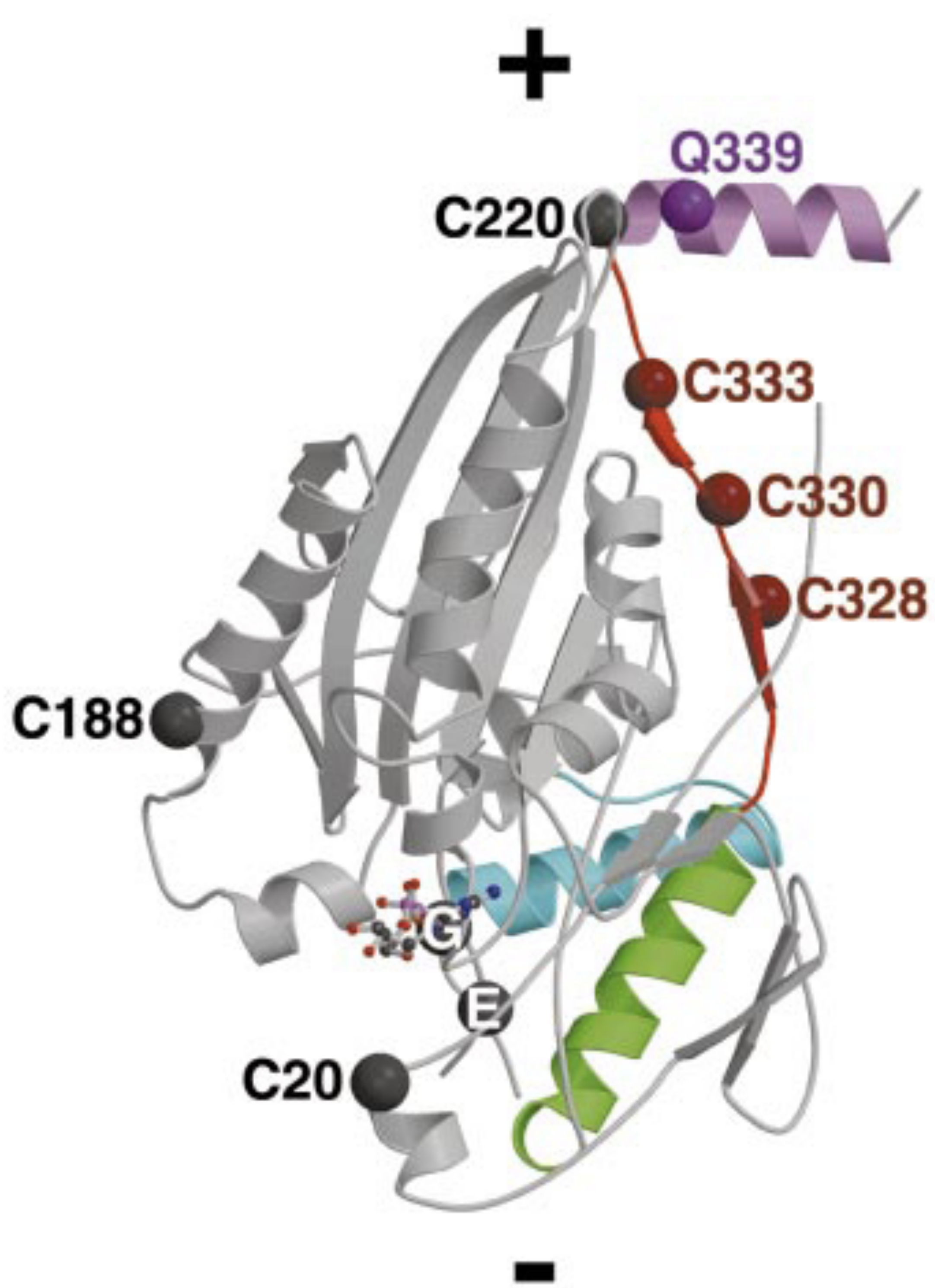




Understanding biochemical cycle (Microscopic rates and binding constant of kinesin)

NECK LINKER DOCKING MODEL

Q: Is the head-neck linker docking model correct (and does it suffice to explain actual stepping)? Does kinesin undertake a conformational "power stroke", or something like it (and if so, how large is it)?



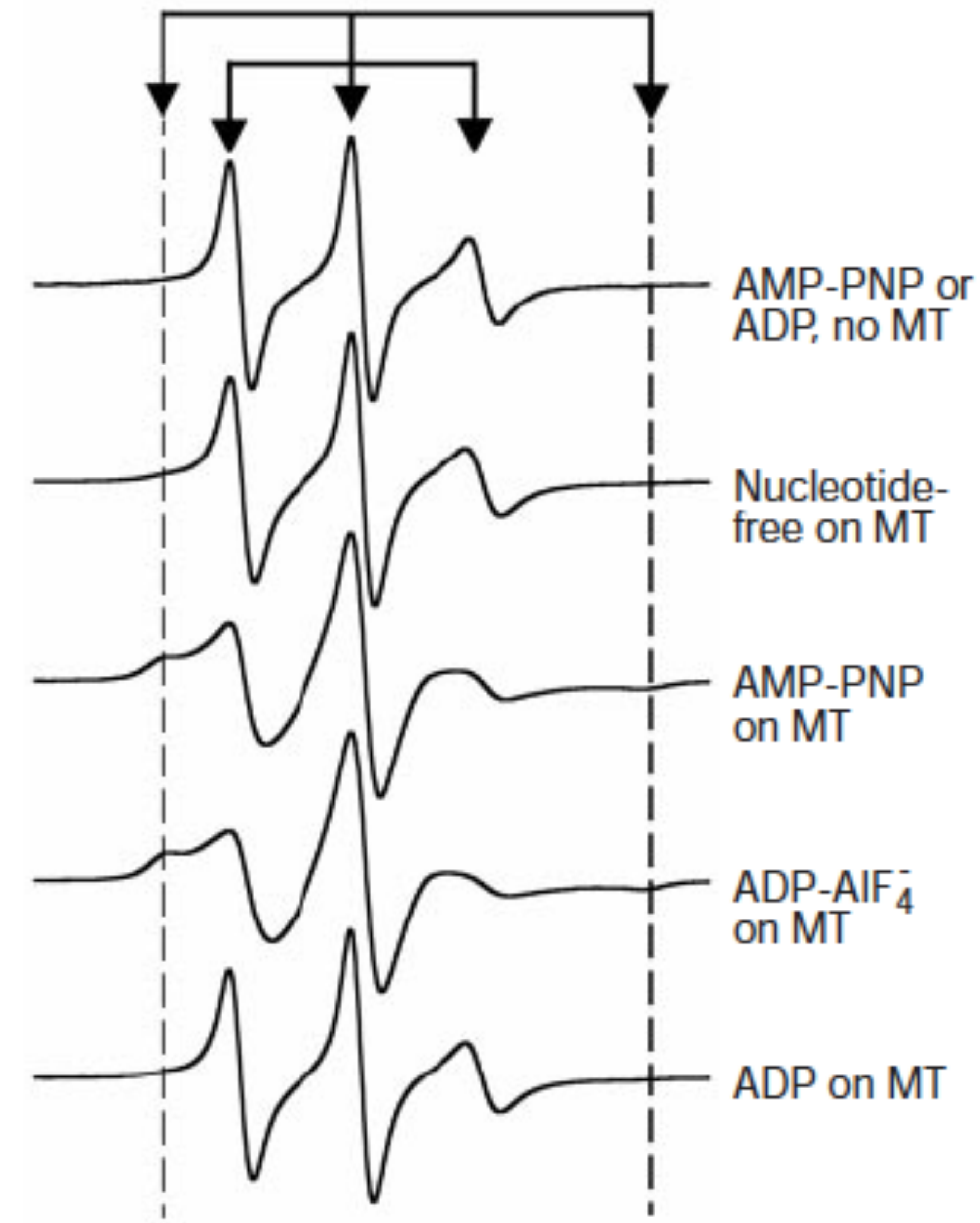
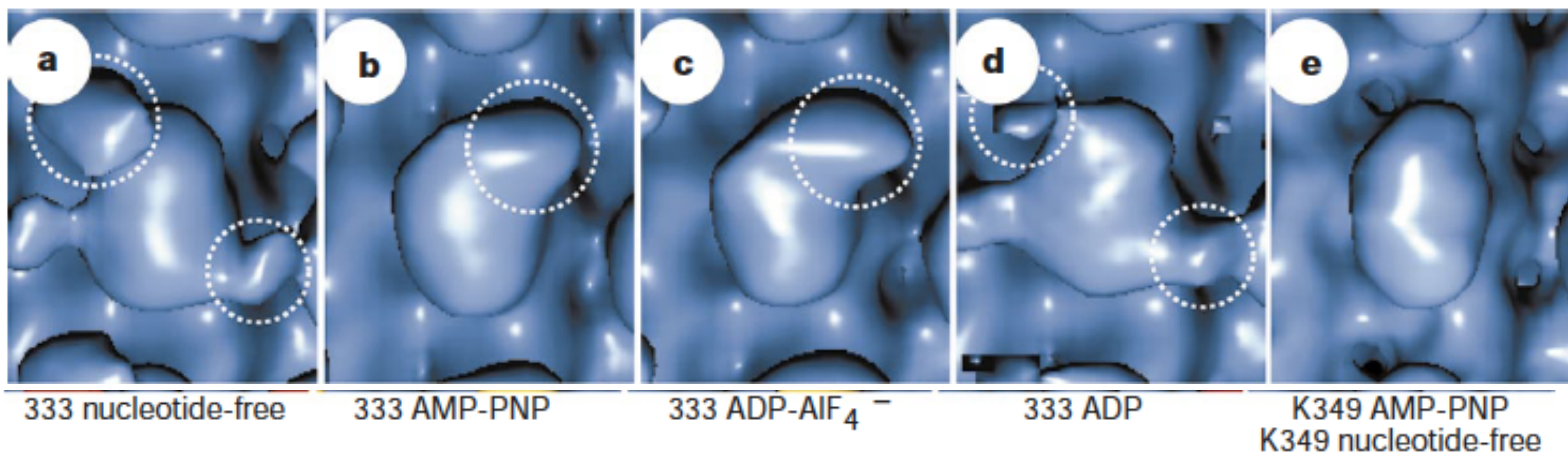


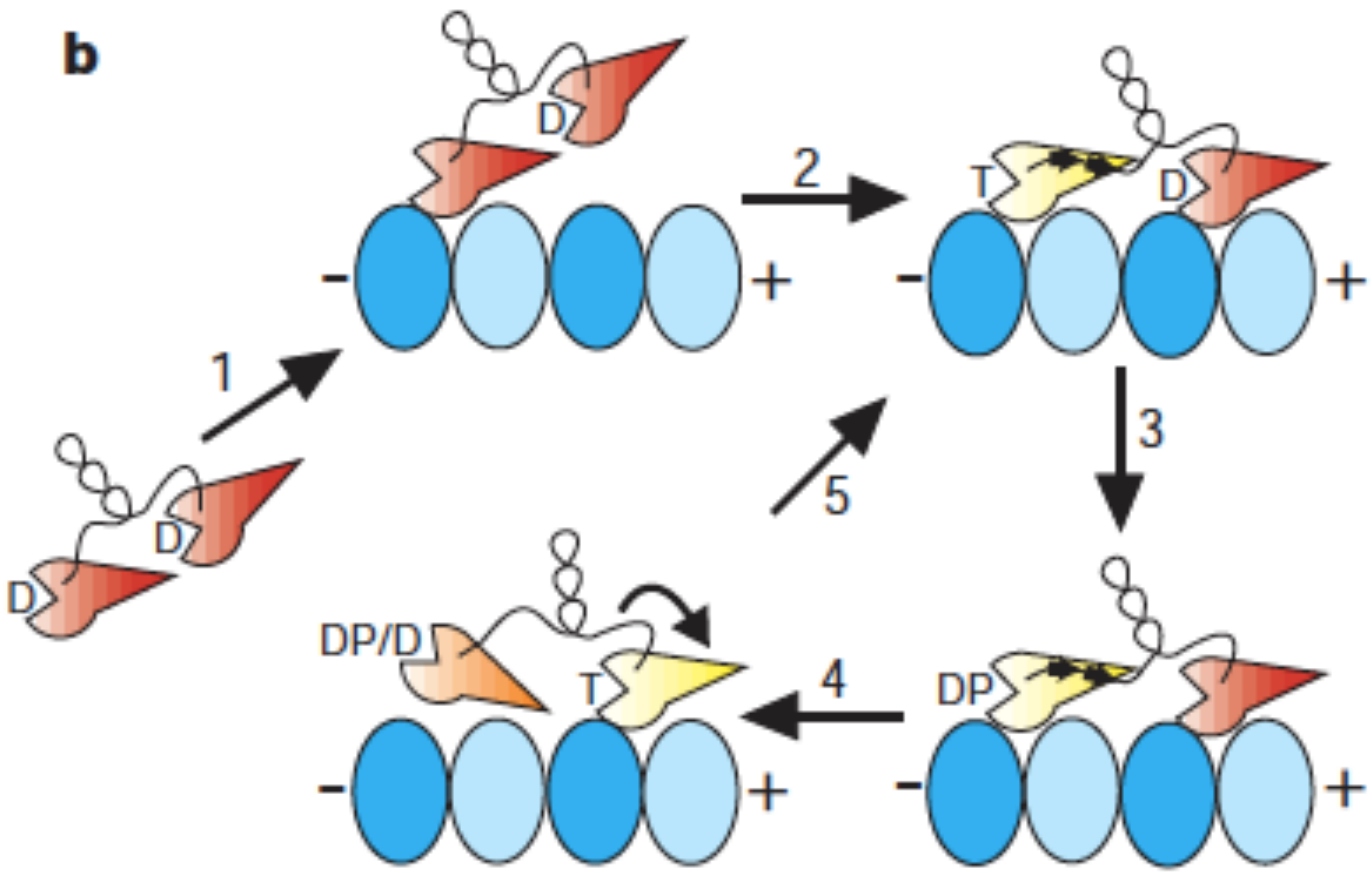
Figure 2 Electron paramagnetic resonance spectra for kinesin C333–MSL in several nucleotide states, both free in solution and bound to microtubules. The two sets of resonance peaks are indicated by arrows. The narrower resonance peaks are indicative of a highly mobile probe, whereas the wider set of resonance peaks (highlighted by the vertical dashed lines) are indicative of restricted mobility and emerge in the triphosphate states on microtubules. This mobility shift can be modelled by a restriction in probe motion from a cone angle of ~120° to 32° (ref. 50).

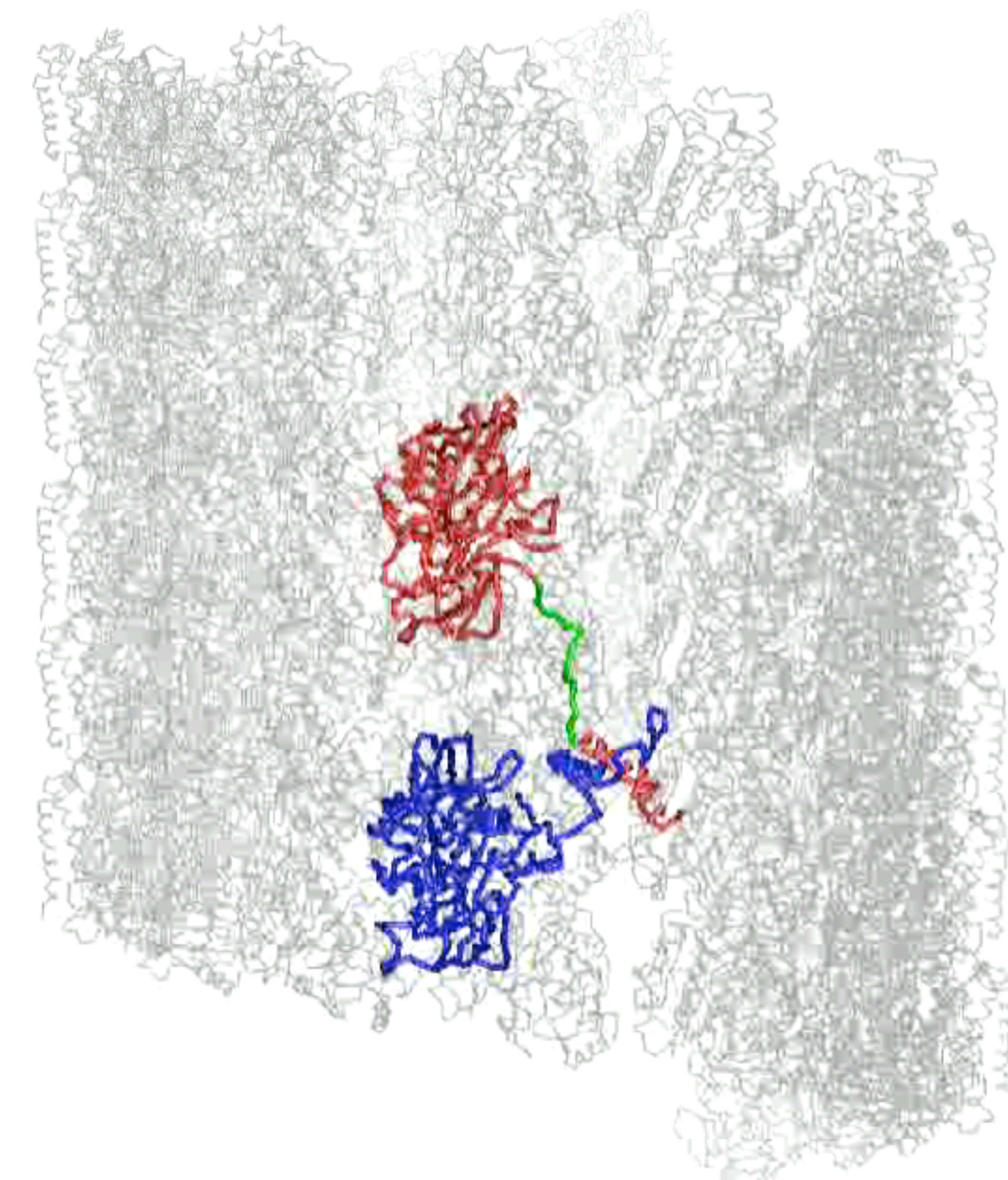
Table 1 FRET between donor (coumarin, CPM) and acceptor (tetramethylrhodamine, TMR) probes attached to the neck linker (C333) and the catalytic core (C220) in wild-type kinesin monomer and two ATP nonhydrolysing mutants

Kinesin (K349–C220, C333)	Nucleotide	MT	Energy transfer (%)
Wild type	ADP	–	87.6 ± 3.3
	AMP-PNP	–	84.4 ± 0.2
	Nucleotide-free	+	76.4 ± 2.8
	AMP-PNP	+	93.4 ± 1.3

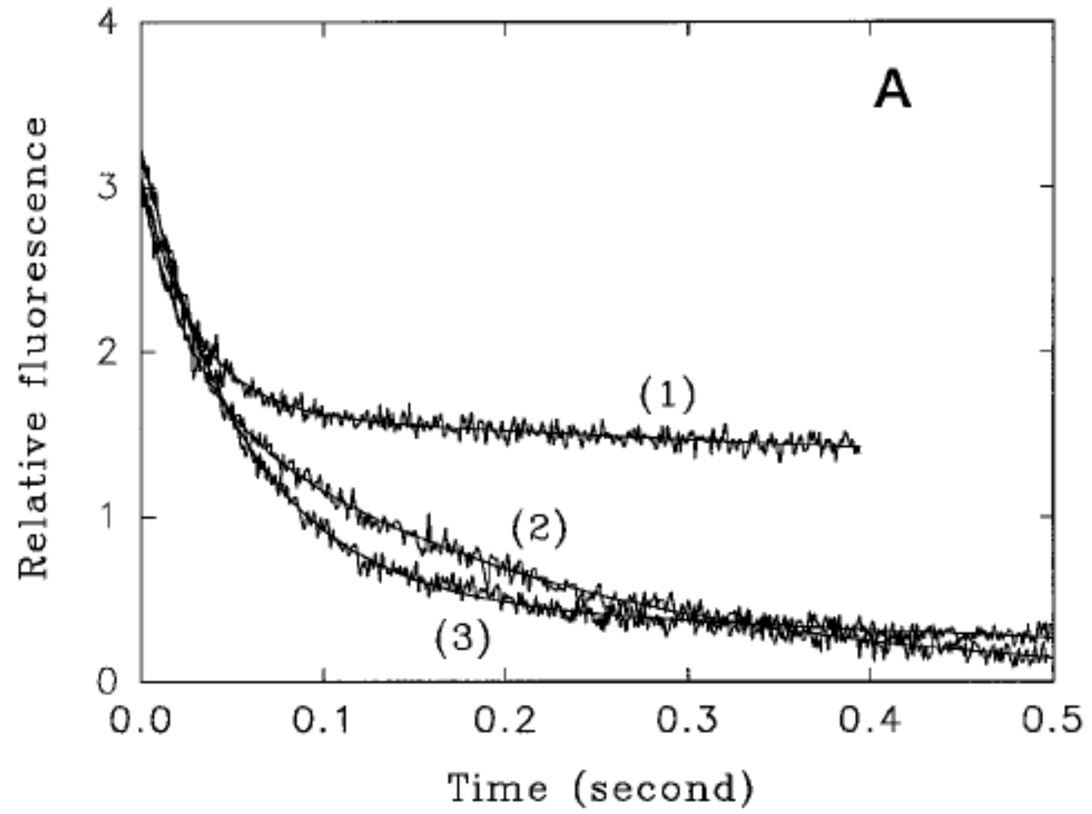


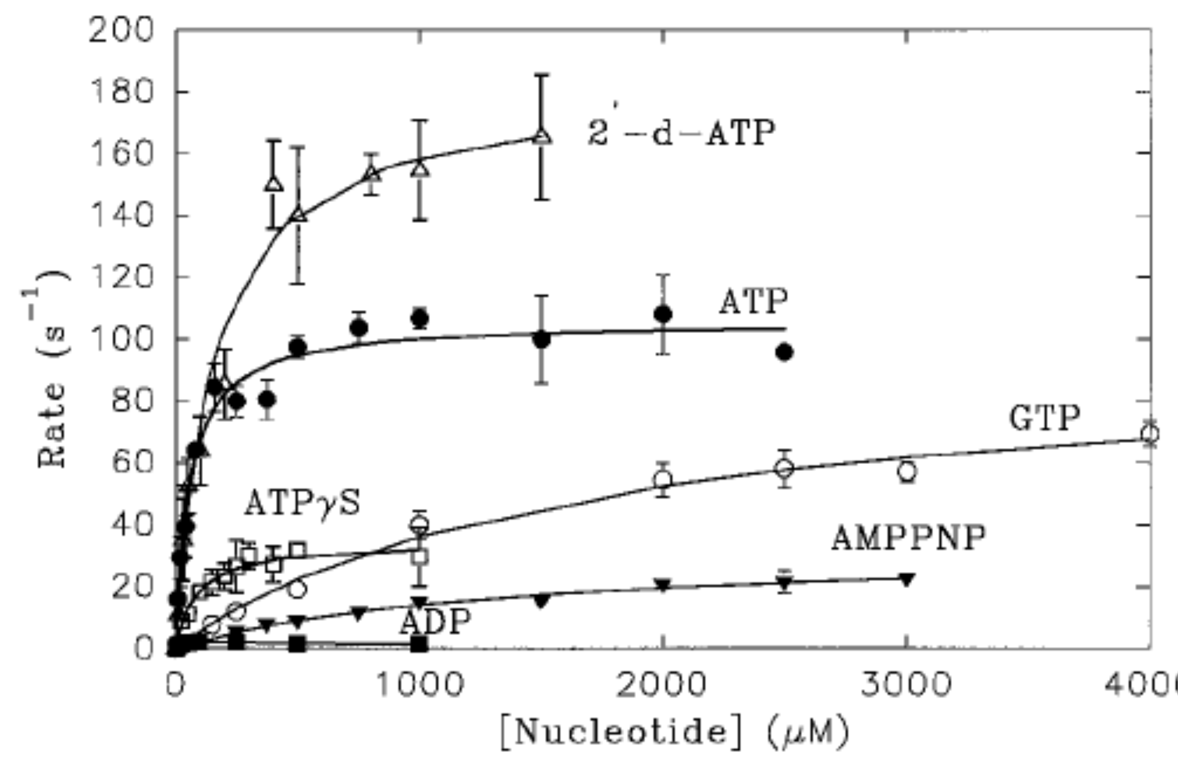
b

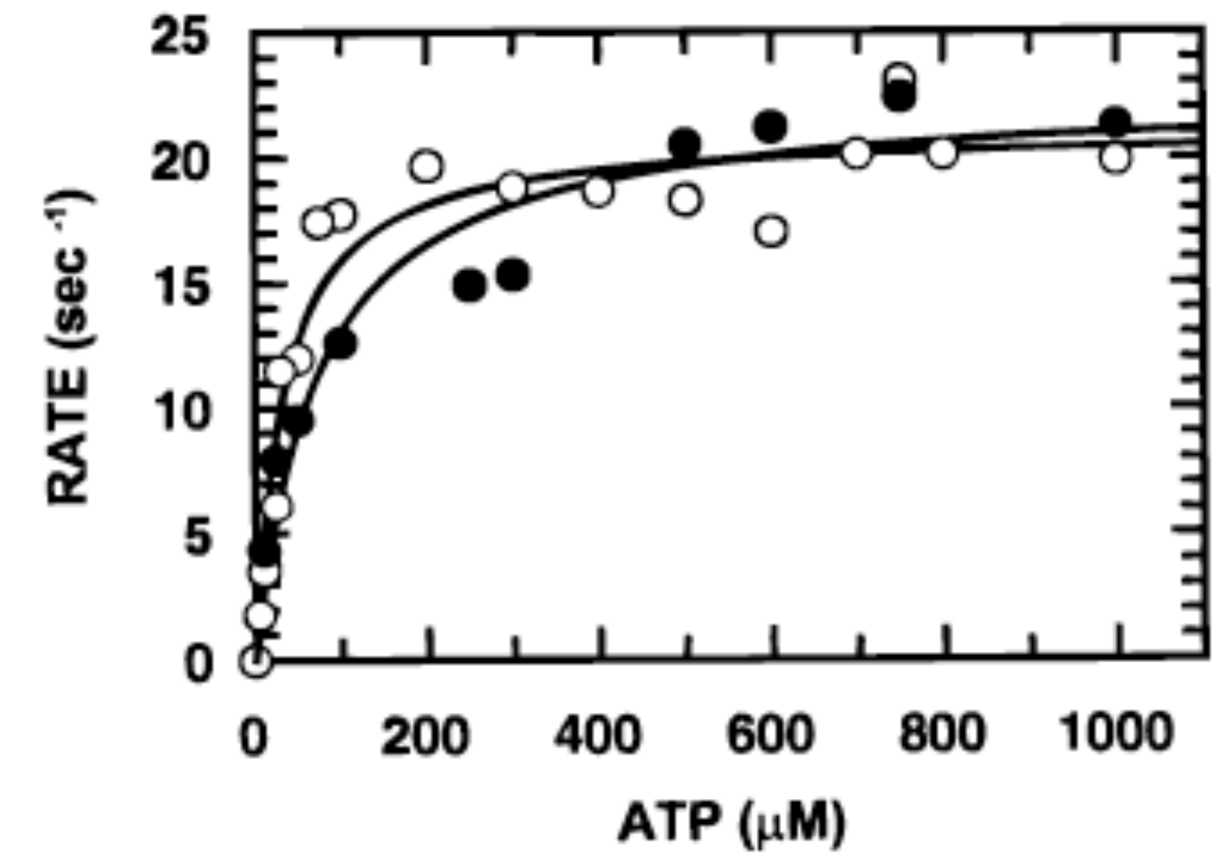




ADP dissociation rate







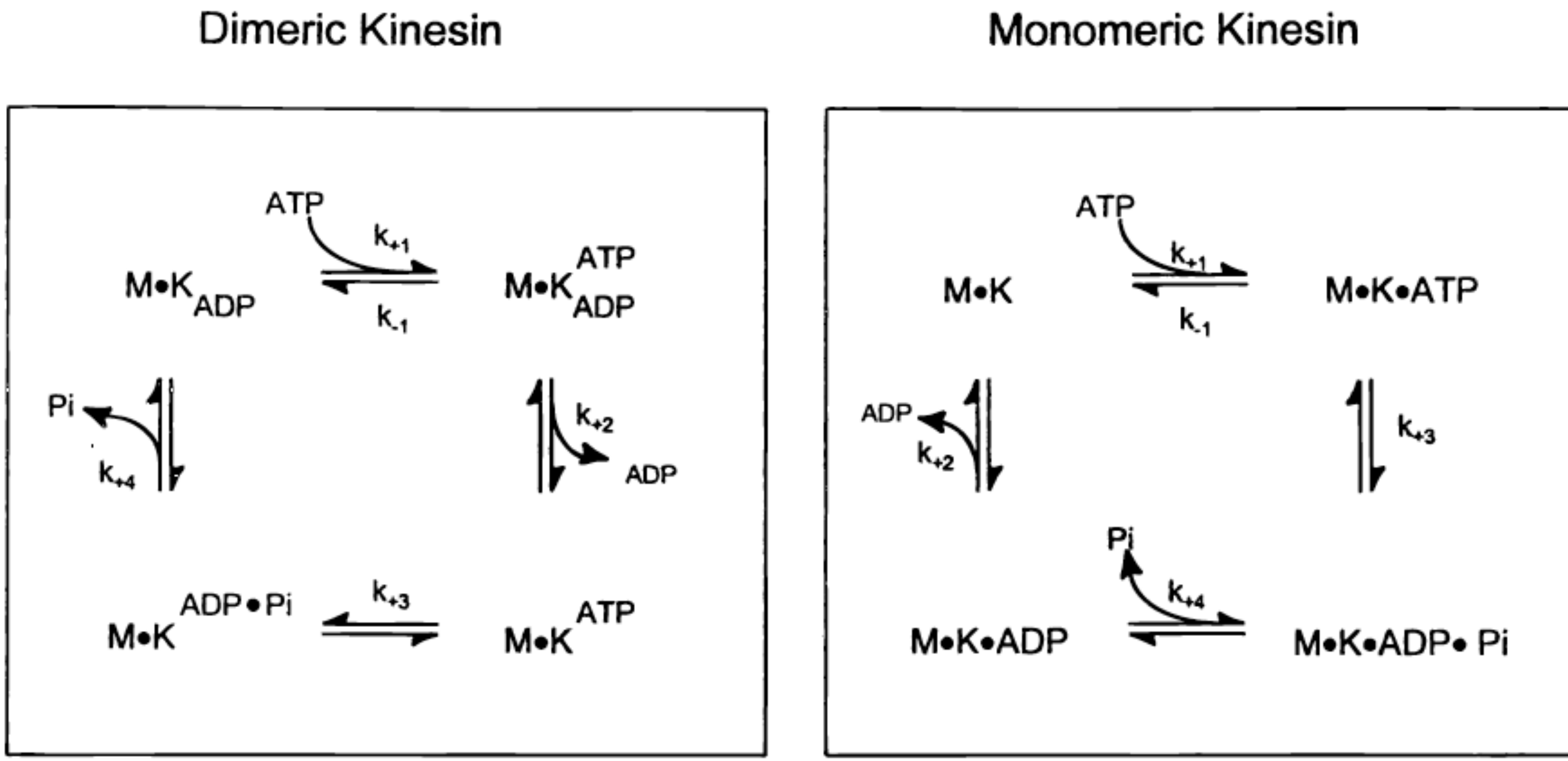
Ma & Taylor *JBC* (1997) 272:724-730

$$M + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M \cdot ATP \xrightarrow{k_{hyd}} M \cdot (ADP \cdot P_i) \xrightarrow{k_{dPi}} M \cdot ADP \xrightarrow{k_{dADP}} M + ADP$$

$$k = \frac{k_{cat}[ATP]}{K_M + [ATP]} = \frac{k_{cat}[ATP]}{\frac{k_1 + k_{cat}}{k_{-1}} + [ATP]}$$

$$k_{cat}^{-1} = k_{hyd}^{-1} + k_{dPi}^{-1} + k_{dADP}^{-1}$$

★ATP binding kinetics, thermodynamics
★Pi release kinetics
★ATP promoted dissociation of MT-kinesin complex
★ADP release kinetics w or w/o MT
★etc ....



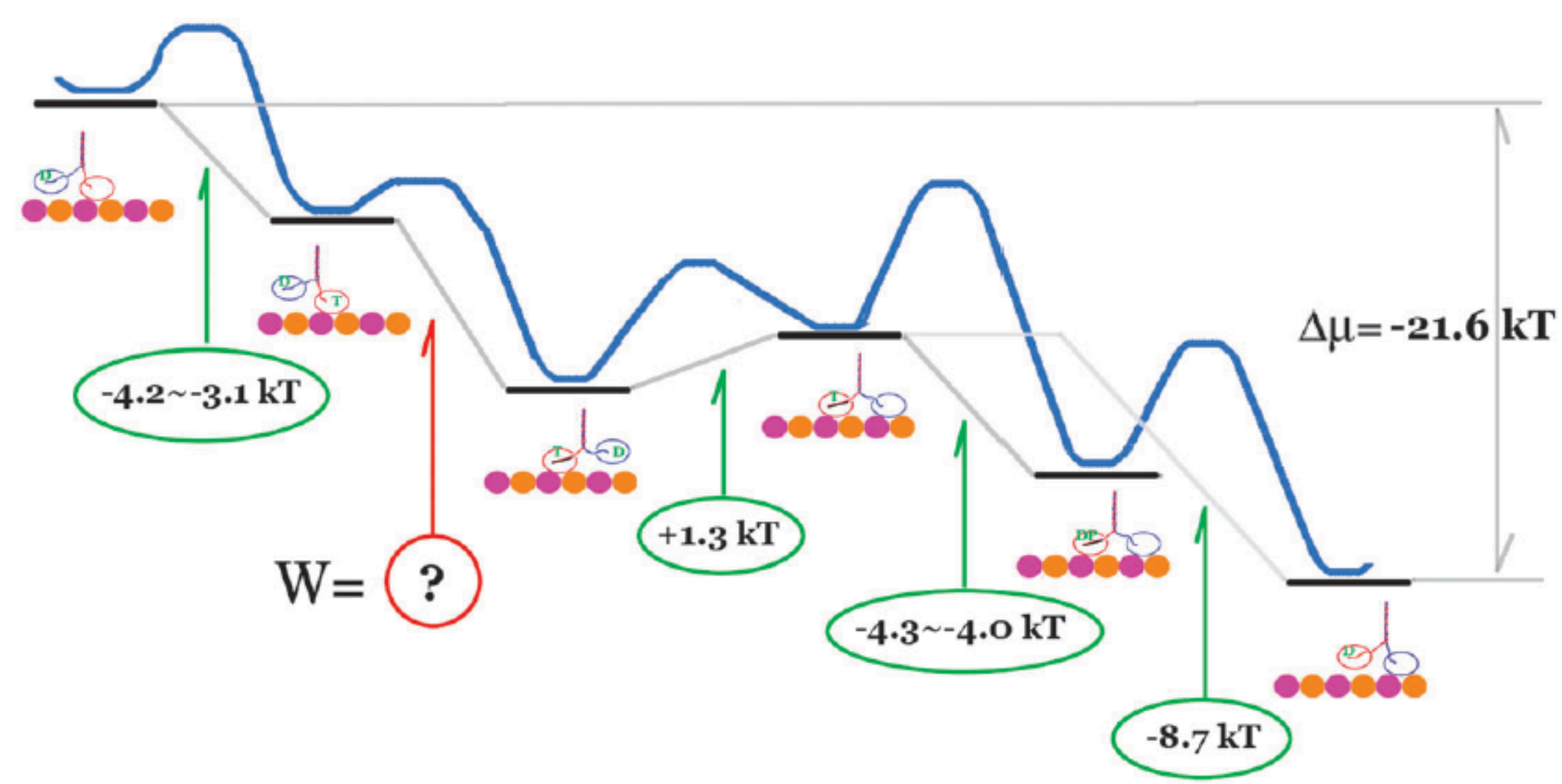
	k_{+1} ($\mu M^{-1}\ s^{-1}$)	k_{-1} (s^{-1})	k_2 (s^{-1})	k_3 (s^{-1})	k_4 (s^{-1})	$k_{cat}{}^{b}$ (s^{-1})
K401	2	71	300	150	50	20
K341	20	200	300	>300	80	80

[a] The scheme depicts the steps in the ATPase pathway for monomeric and dimeric kinesin with rate constants defined in the table. For dimeric kinesin, one turnover of ATP requires one and one-half turns of the ATPase cycle shown. [b] Maximum steady-state rate of ATP hydrolysis per enzyme active site.

Table 2 Rate and equilibrium constants for the microscopic steps along the cycle

	Rate constant	Equilibrium constant
[a] $k_T^+ (\equiv k_T)$	$k_T^o = 2.0 \pm 0.8\ \mu M^{-1}\ s^{-1}$ [5]	$K_T = k_T^o / k_{-T} = (35\ \mu M)^{-1}$ [5]
[b] $k_T^- (\equiv k_{-T})$	$k_{-T} = 71 \pm 9\ s^{-1}$ [5]	
[c] $k_S^+ (\equiv k_S)$	$k_S \gtrsim (100\ \mu s)^{-1}$	No data
[d] $k_S^- (\equiv k_{-S})$		
[e] $k_{-D}^+ (\equiv k_{-D})$	$k_{-D[L]} = 75\text{–}100\ s^{-1}$ [7] $k_{-D[T]} = 1\ s^{-1}$ [7]	$K_{D[L]} = k_D^o / k_{-D} = 5 \times 10^4\ M^{-1}$ [7] $K_{D[T]} = k_D^o / k_{-D} = 5 \times 10^6\ M^{-1}$ [7]
[f] $k_{-D}^- (\equiv k_D)$	No data	
[g] $k_h^+ (\equiv k_h)$	$k_h > 100 \pm 30\ s^{-1}$ [3]	$K_h < 39$
[h] $k_h^- (\equiv k_{-h})$	$k_{-h} = 1.3\ s^{-1}$	
[i] $k_{-P}^+ (\equiv k_{-P})$	$k_{-P} = 50\ s^{-1}$	No data
[j] $k_{-P}^- (\equiv k_P)$	No data	
[k] $k_{h,-P}$	$k_{h,-P} = 100\text{–}300\ s^{-1}$ $k_{-h,P} = 34\ M^{-1}\ s^{-1}$ [2]	$K_{h,-P} = 200\ s^{-1} / 34\ M^{-1}\ s^{-1} = 6\ M$ [2]

[a] Bi-molecular rate constant for ATP binding to the nucleotide free kinesin head. [b] Rate constant for ATP dissociation from the ATP bound kinesin head. [c] Rate constant for kinesin stepping. [d] Rate constant for kinesin backstepping. [e] Rate constant for ADP dissociation. [f] Rate constant for ADP re-binding. [g] Rate constant for hydrolysis at the catalytic site. The free energy change for ATP hydrolysis ATP $\rightleftharpoons$ ADP + P_i under standard aqueous conditions (aq., 1 atm, 25 °C) is $\Delta\mu^o = -12\ k_BT$. [h] Rate constant for ATP synthesis. [i] Rate constant for phosphate dissociation. [j] Rate constant for phosphate re-binding. [k] Rate constant for hydrolysis followed by phosphate release.



Nucleotide state at the catalytic site of kinesin determines the binding affinity to the MT and the neck-linker conformation

	MT	Neck-linker
K.Φ	Strongly bound	Disordered (unzippered)
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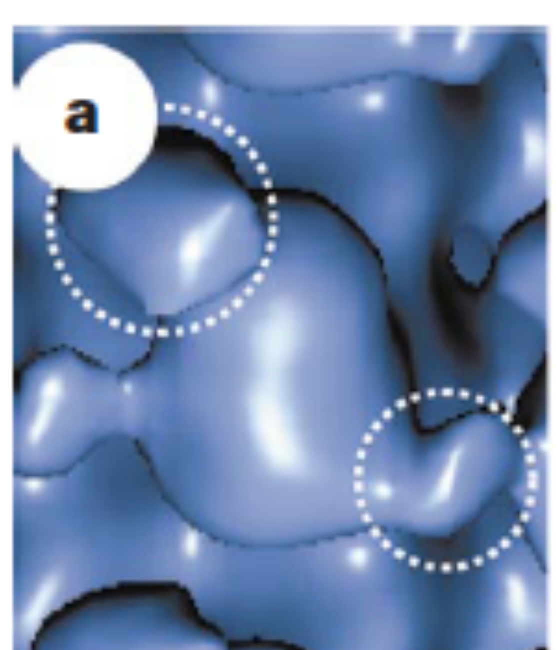
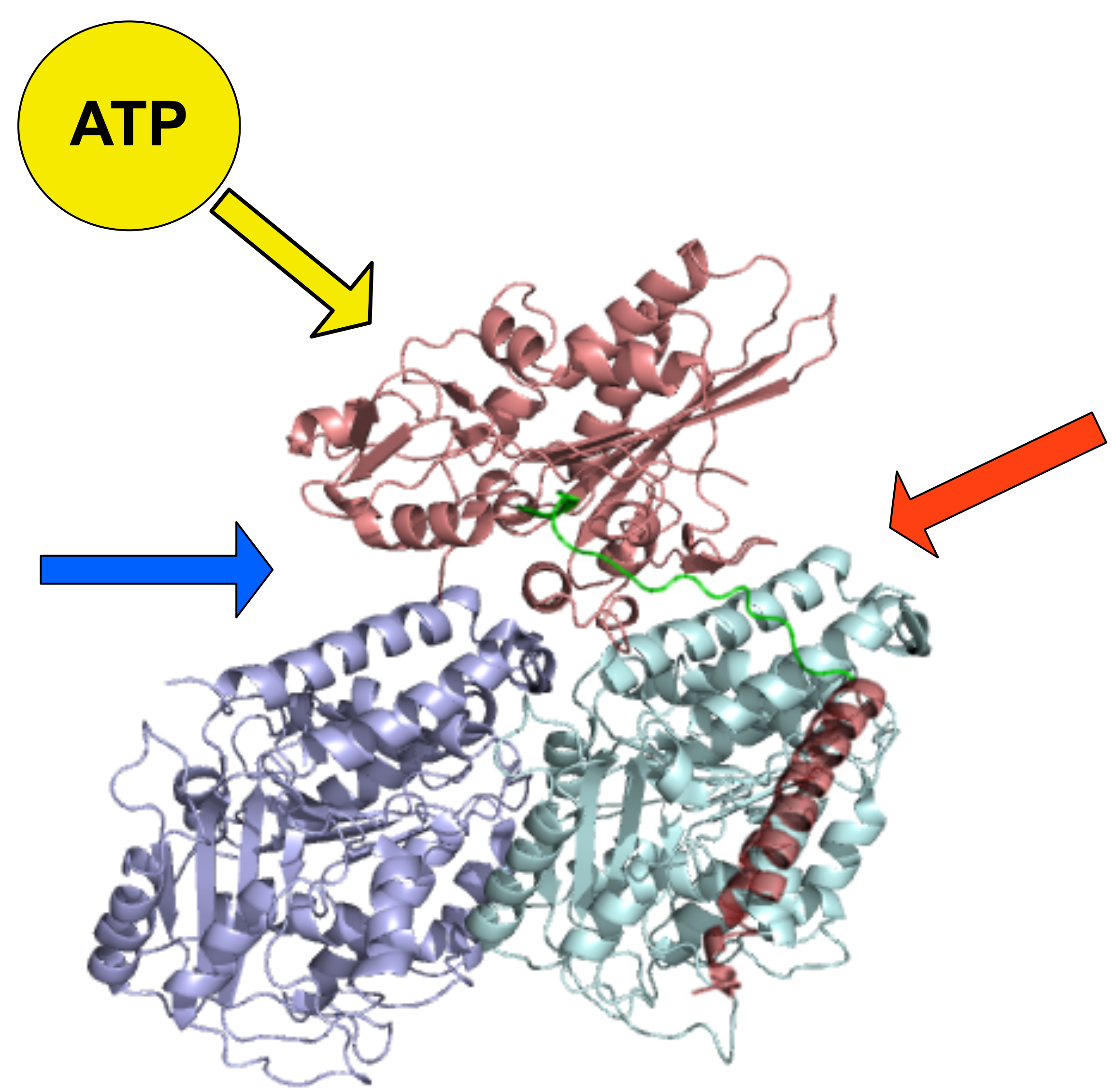
Cryo-EM



Rice et al (1999) Nature



From a number of ensemble and single molecule experiments....

(Asbury, Block, Cross, Gilbert, Hackney, Hirokawa, Higuchi, Howard, Ishiwata, Johnson, Selvin, Spudich, Taylor, Vale, Vissher, Yanagida, ... and more)

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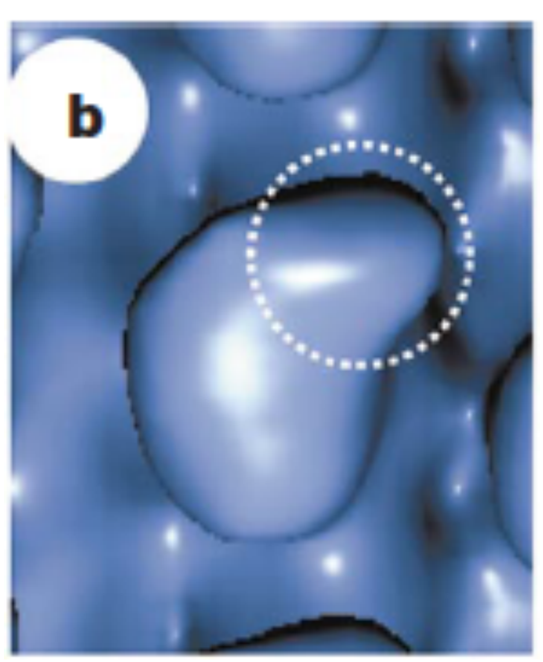

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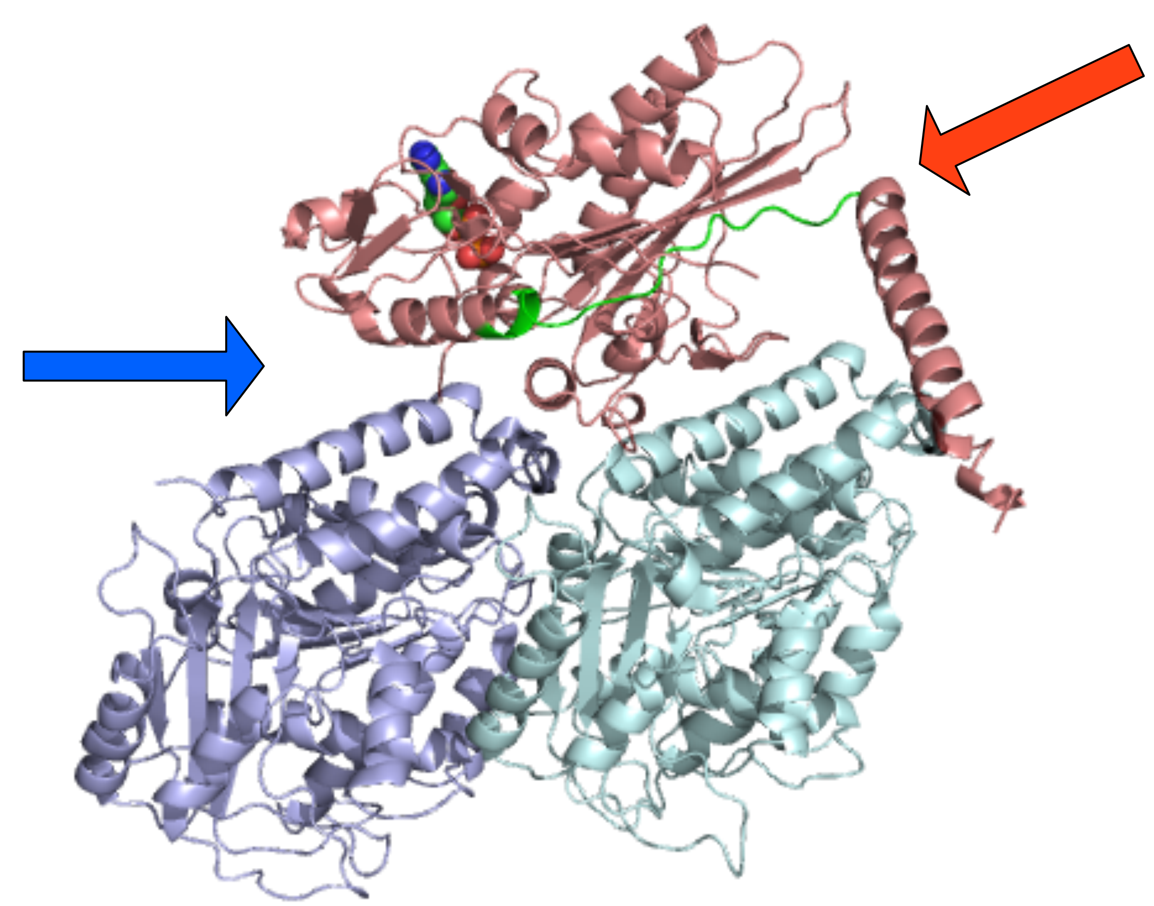

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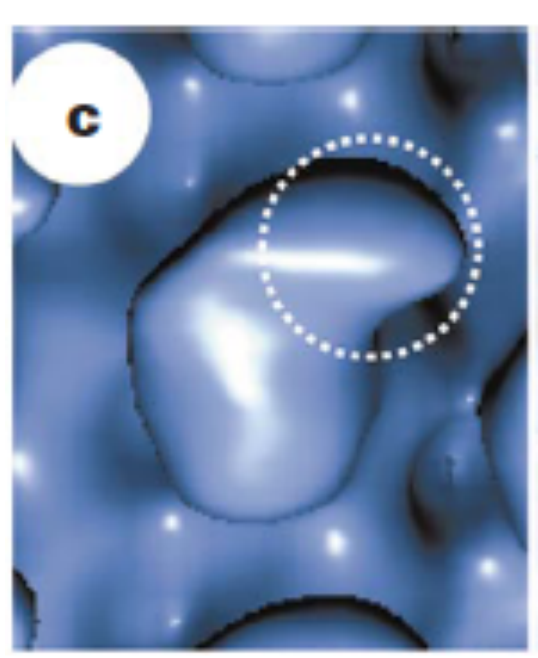

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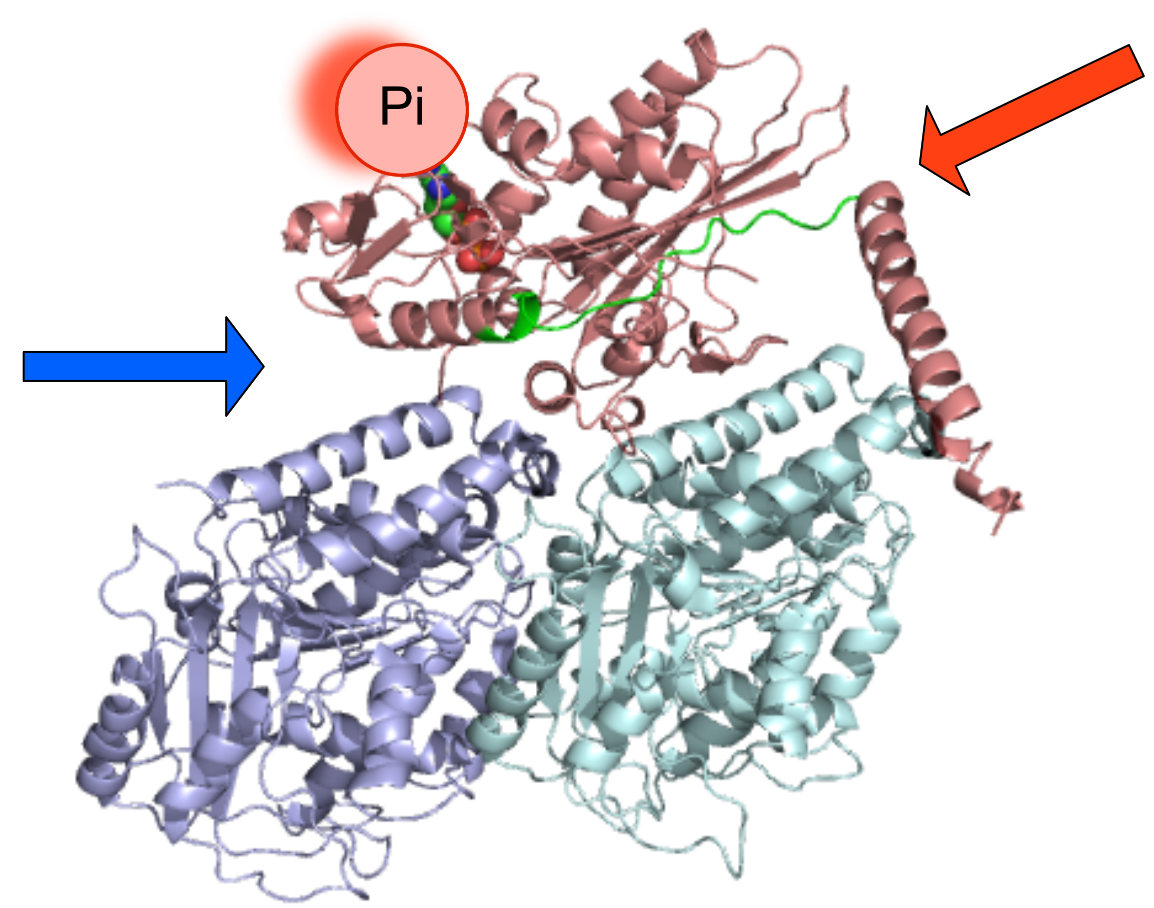

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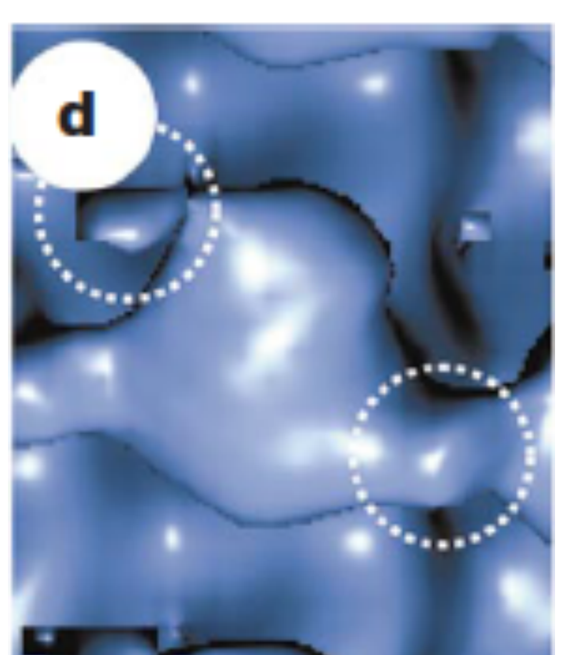

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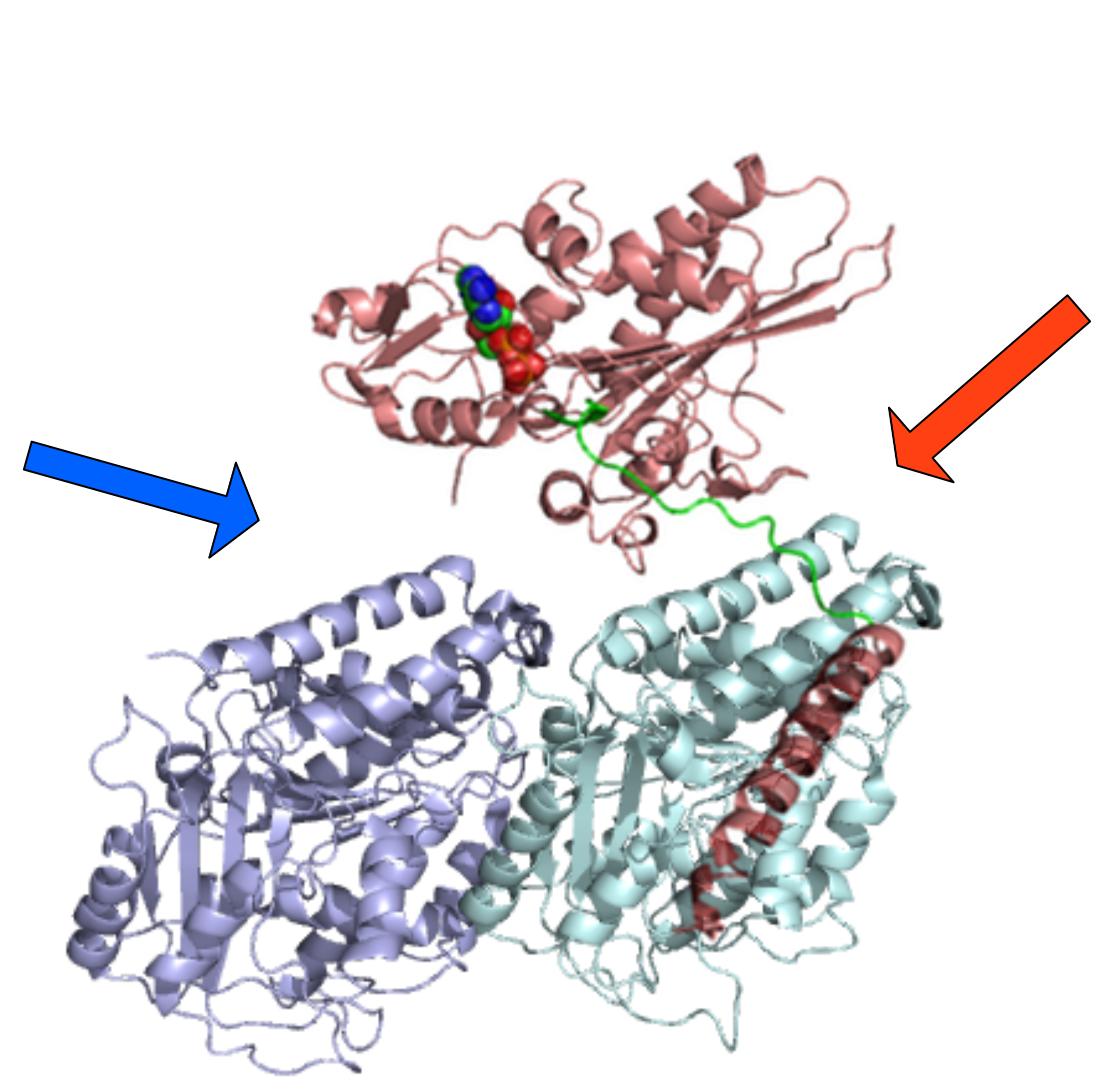

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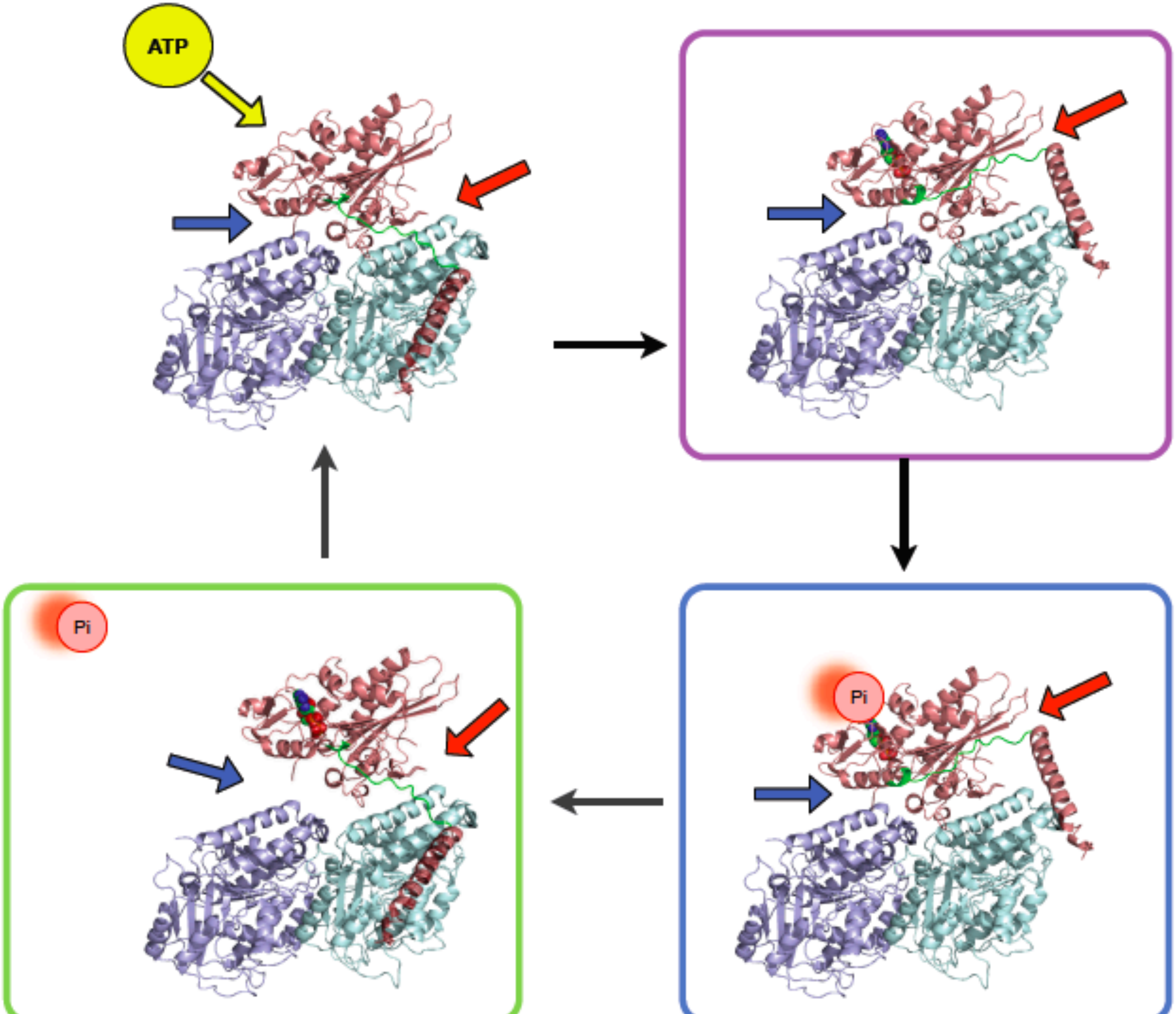
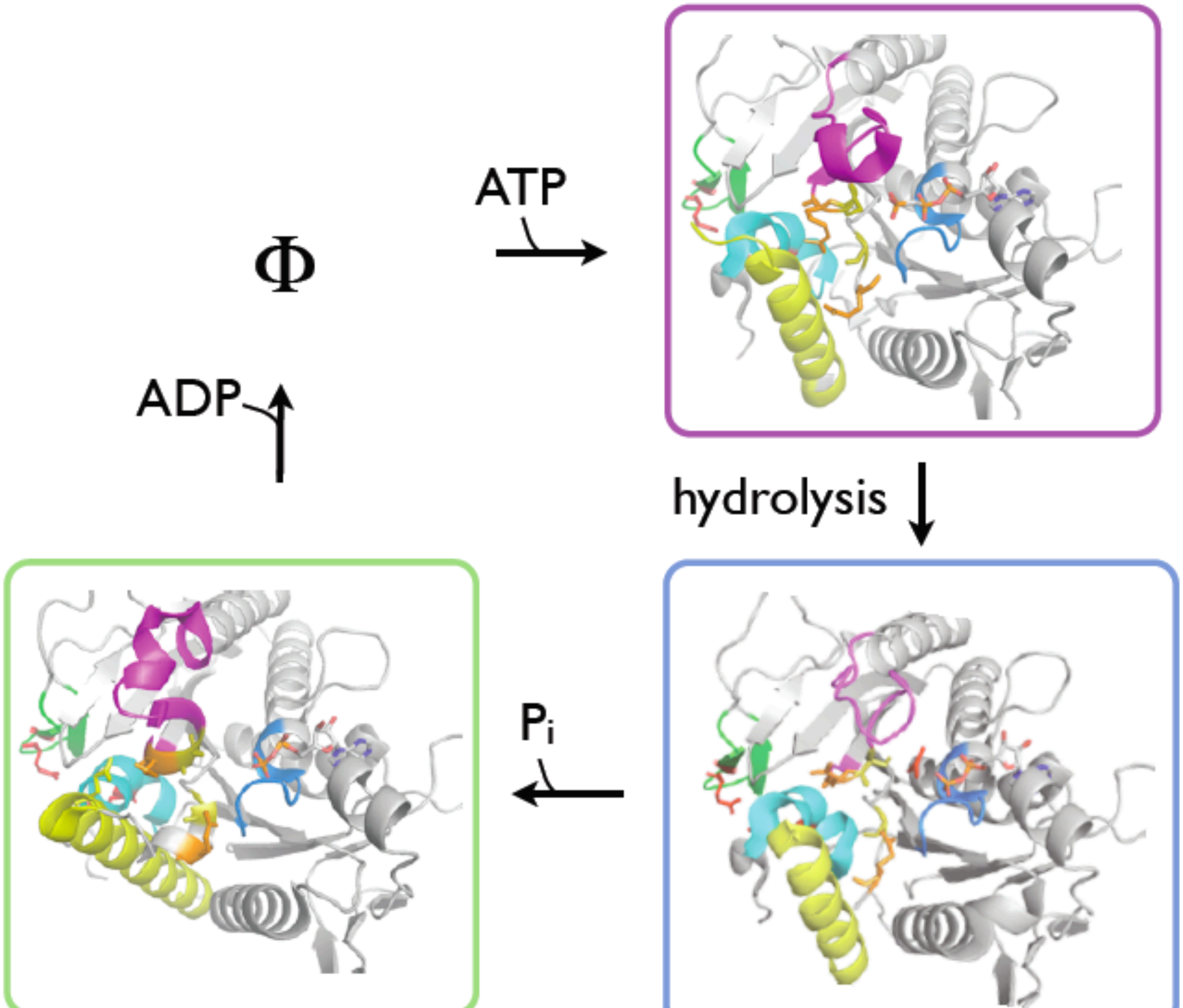
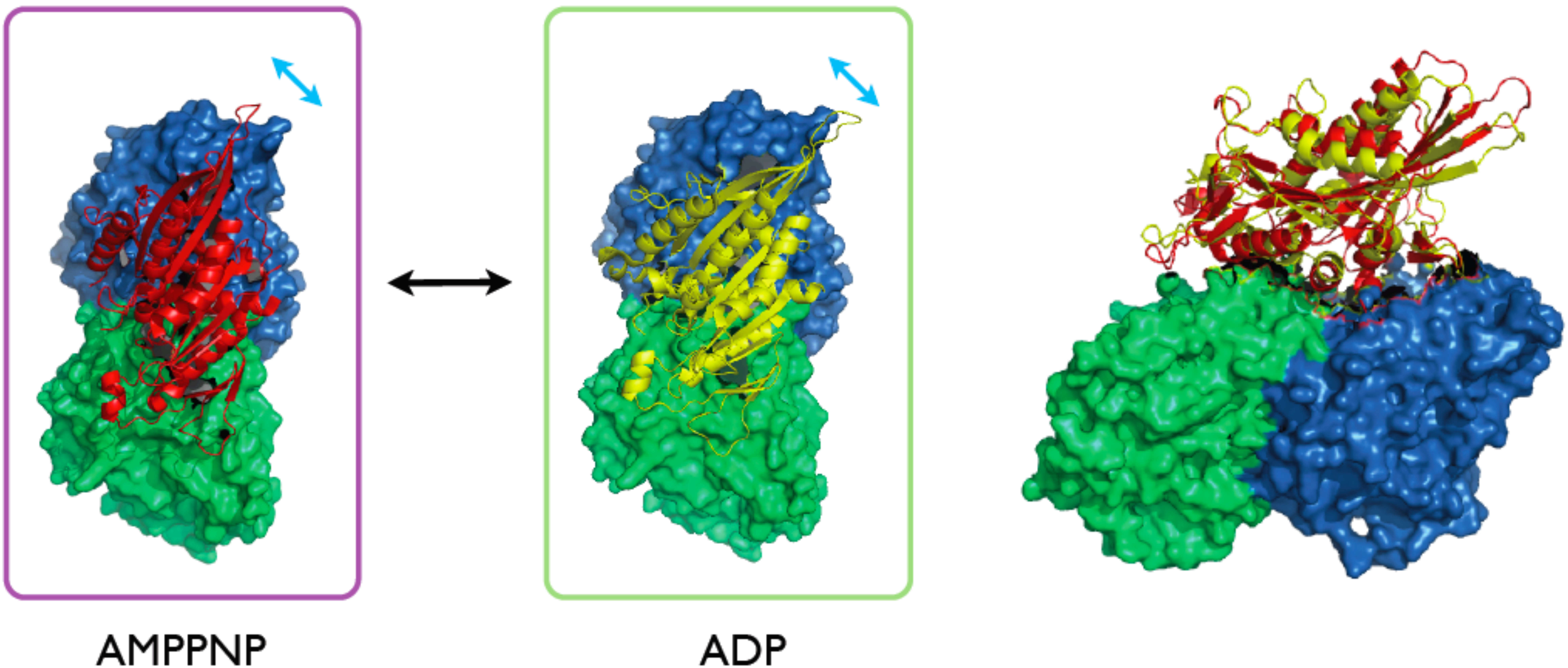

ATP
Pi
Pi



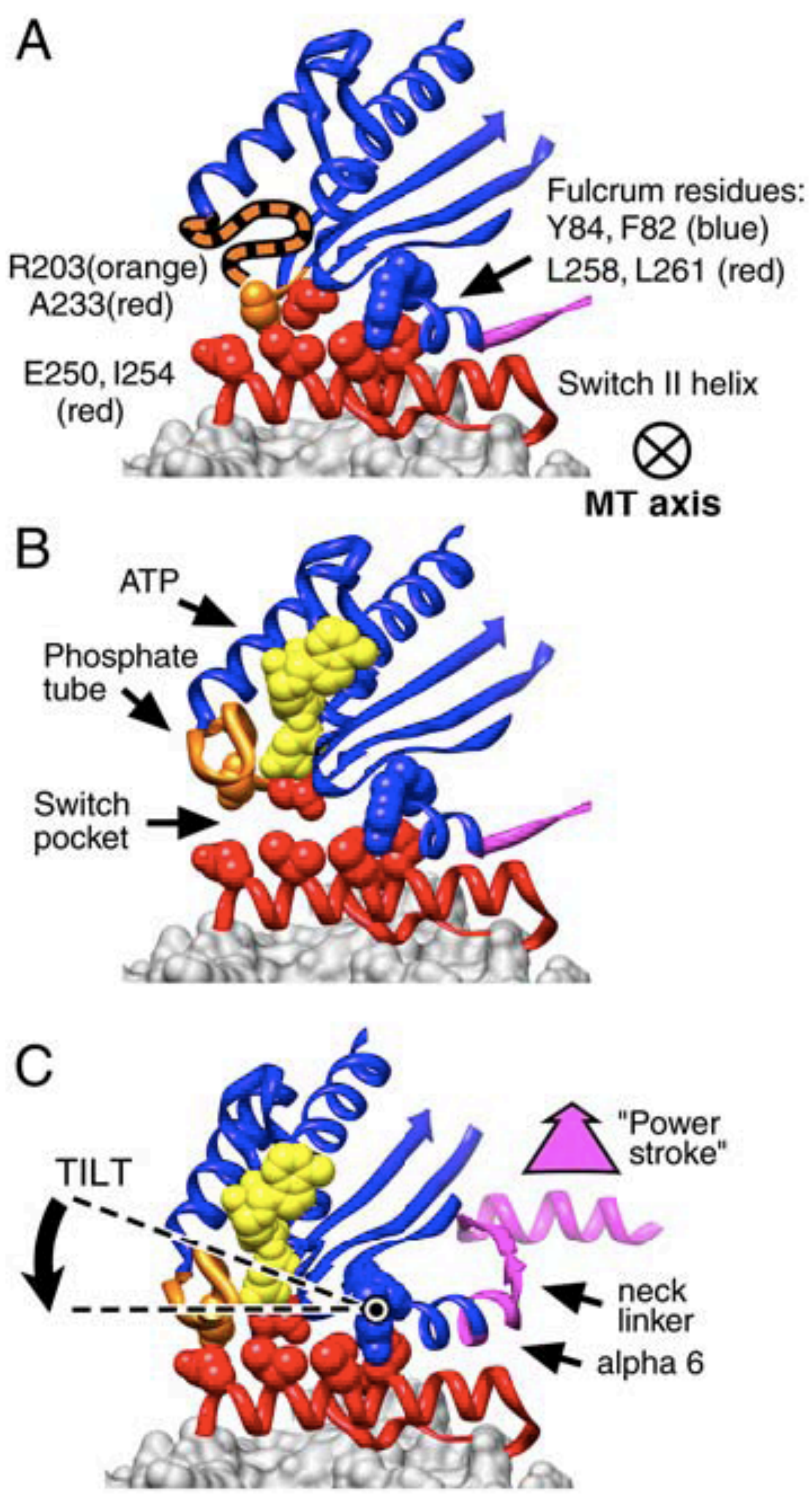
Φ
ATP
ADP
hydrolysis
P_i

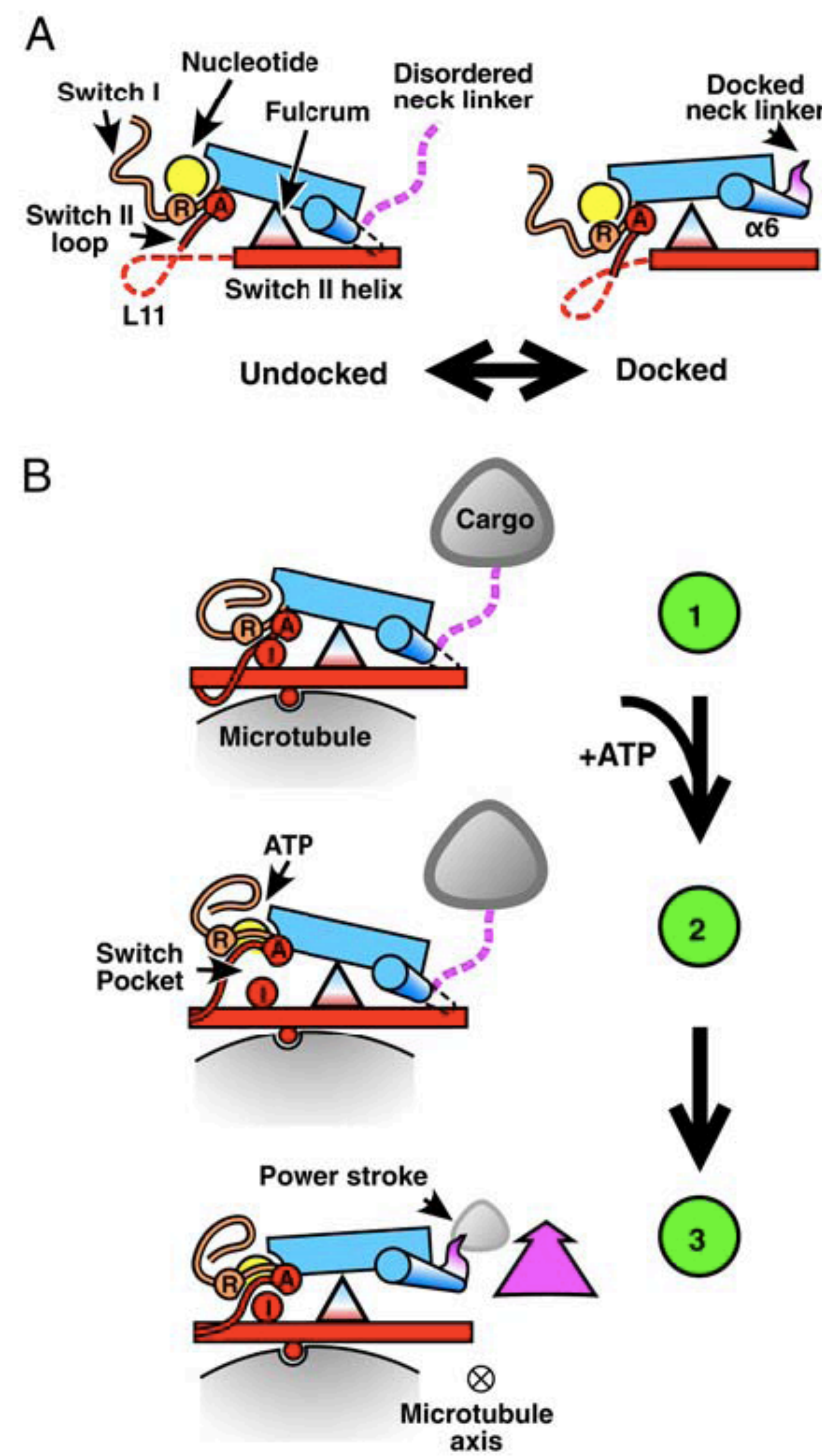

AMPPNP
ADP

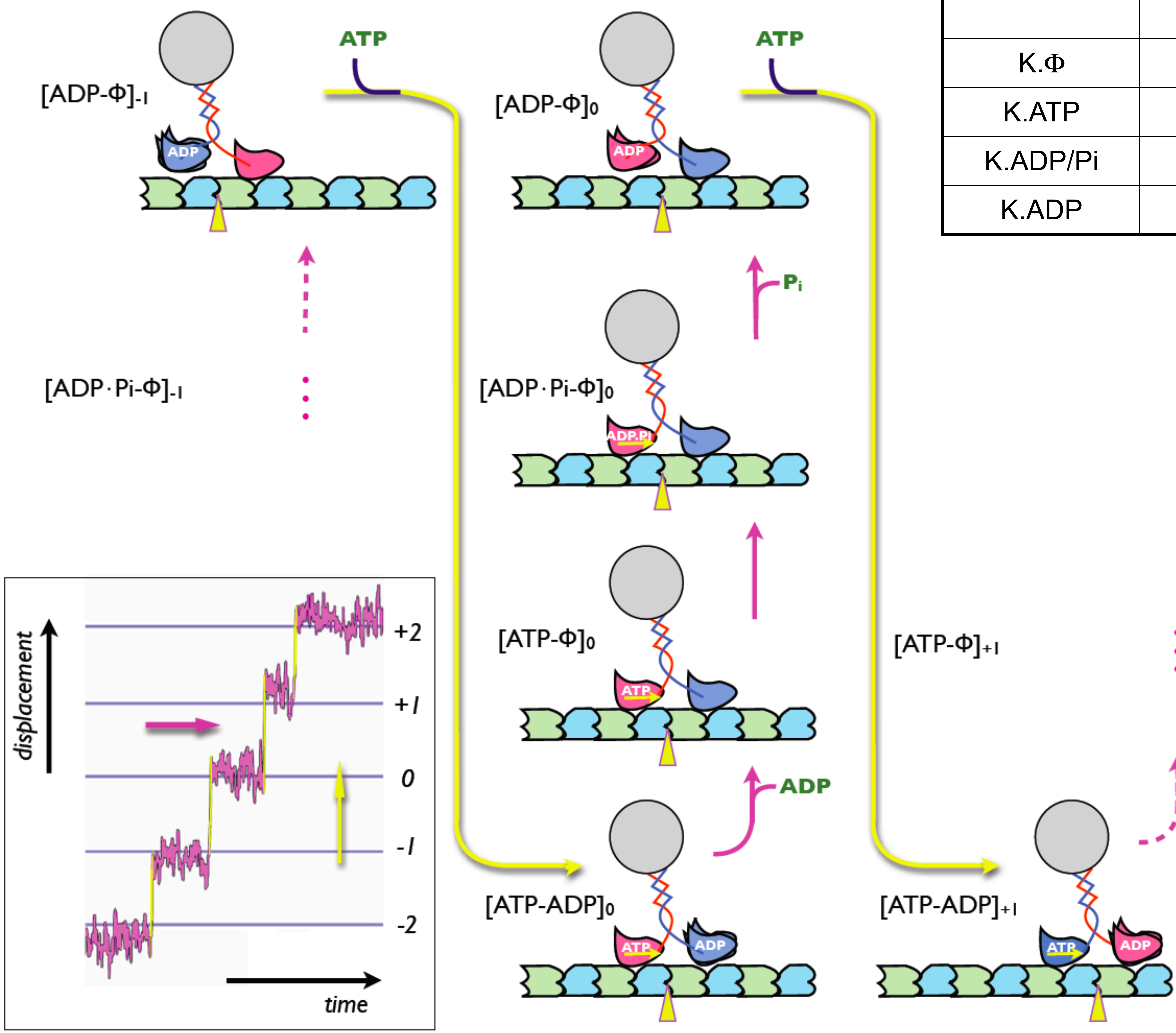
An atomic-level mechanism for activation of the kinesin molecular motors

Charles V. Sindelar[1,2] and Kenneth H. Downing

PNAS | March 2, 2010 | vol. 107 | no. 9 | 4111–4116







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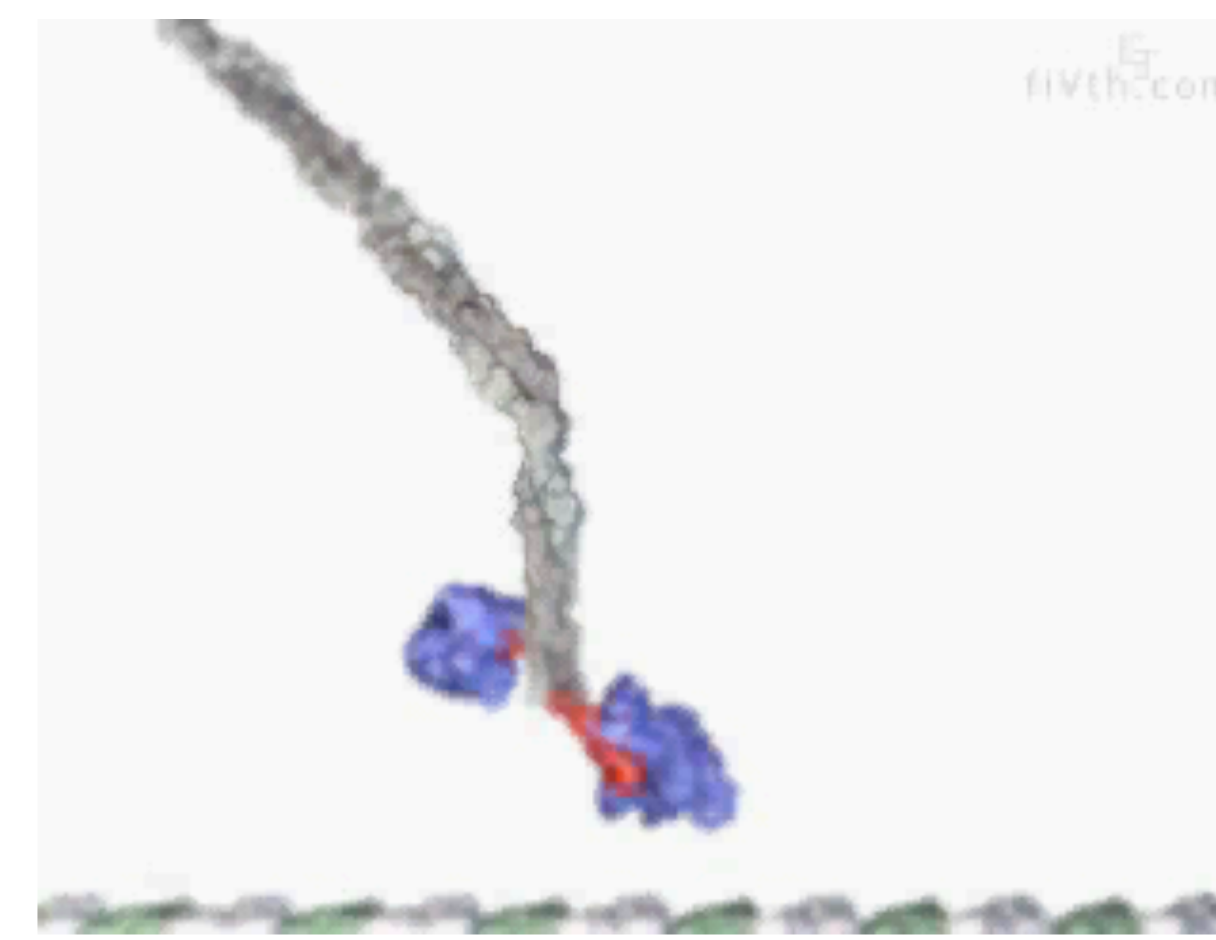
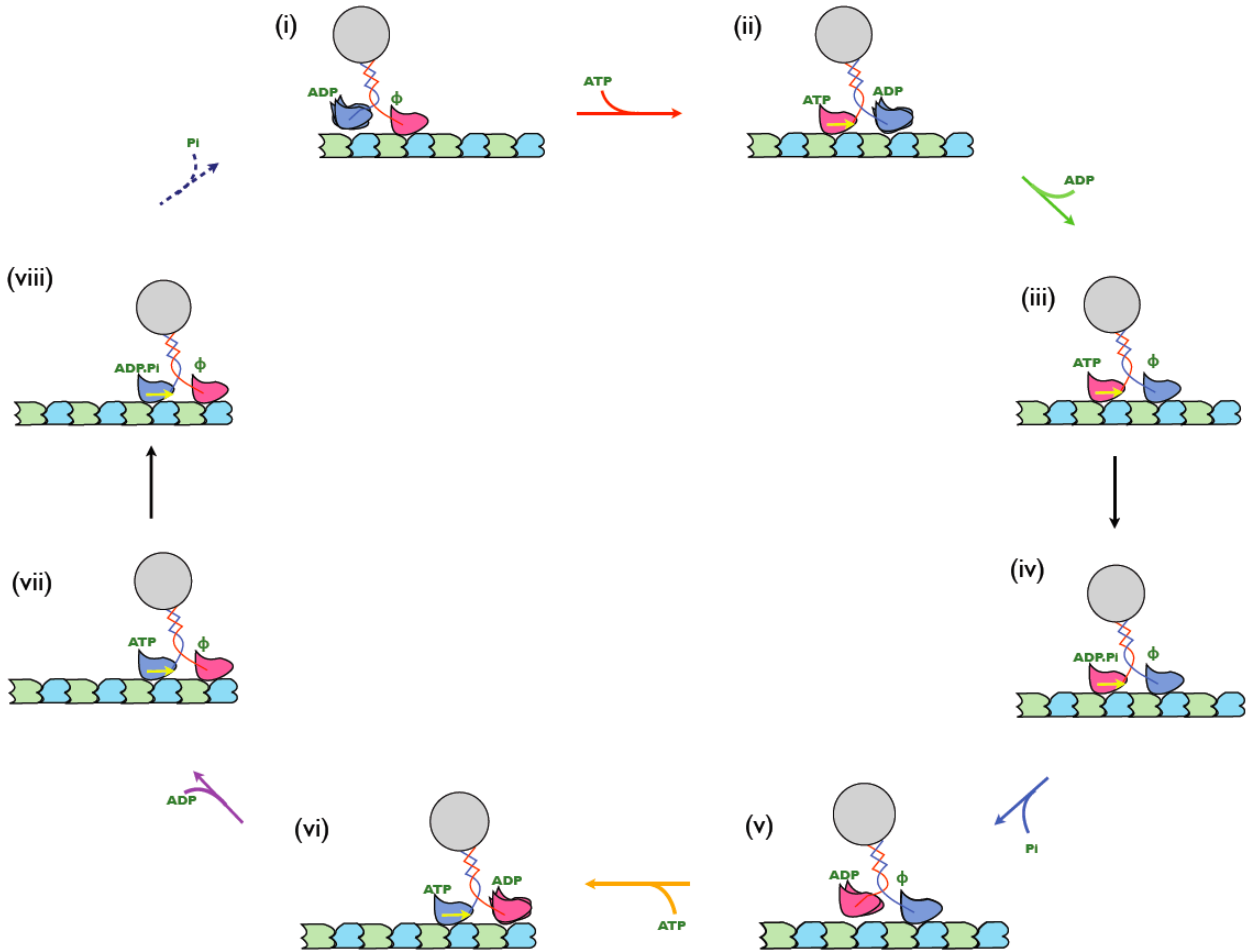
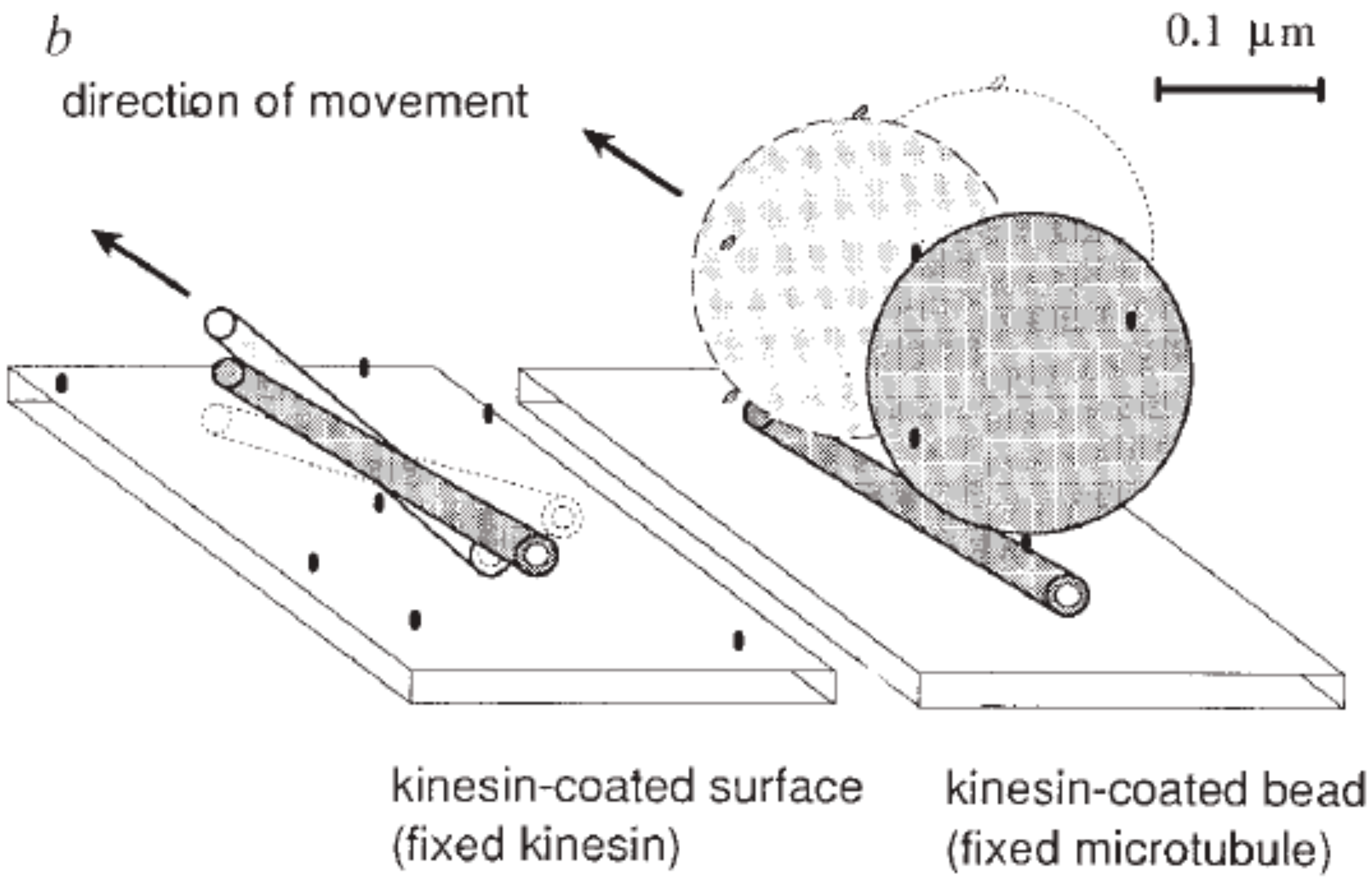




(i)
ADP
ϕ
ATP
(ii)
ATP
ADP
ADP
(iii)
ATP
ϕ
(iv)
ADP.Pi
ϕ
Pi
(v)
ADP
ϕ
ATP
(vi)
ATP
ADP
ADP
(vii)
ATP
ϕ
(viii)
ADP.Pi
ϕ
Pi

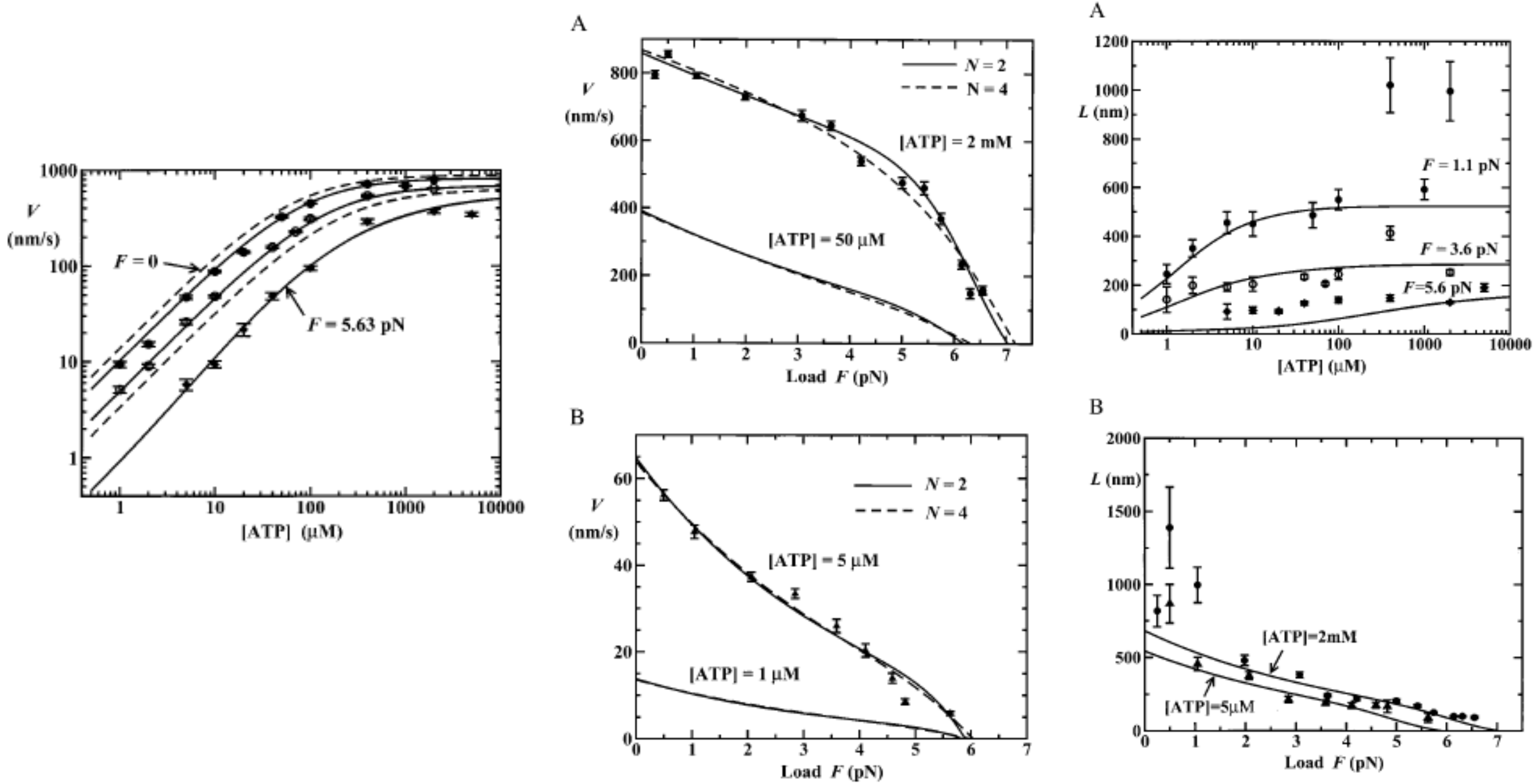


Gliding assay Bead assay

Monitor the response of kinesin motors under ATP concentration and/or force perturbation

Motor Velocity

Run Length



Questions from Biophys. J. (2007) 92: 2986-2995

- Does kinesin take substeps? If so, over what time and distnace scales?
- What's the kinesin walking pattern, and what do we learn about its mechanics from this? (sym. HoH, asym HoH, inchworm)
- How do the two kinesin heads mange to stay out of phase with one another during the stepping cycle (i.e., how are they "gated")?
- Where in the kinesin biochemical pathway is forward motion produced?
- Is the bacstepping cycle a reversal of the forward cycle, and does kinesin generate ATP under super-stall loads that force it to move backward?

Questions from Biophys. J. (2007) 92: 2986-2995

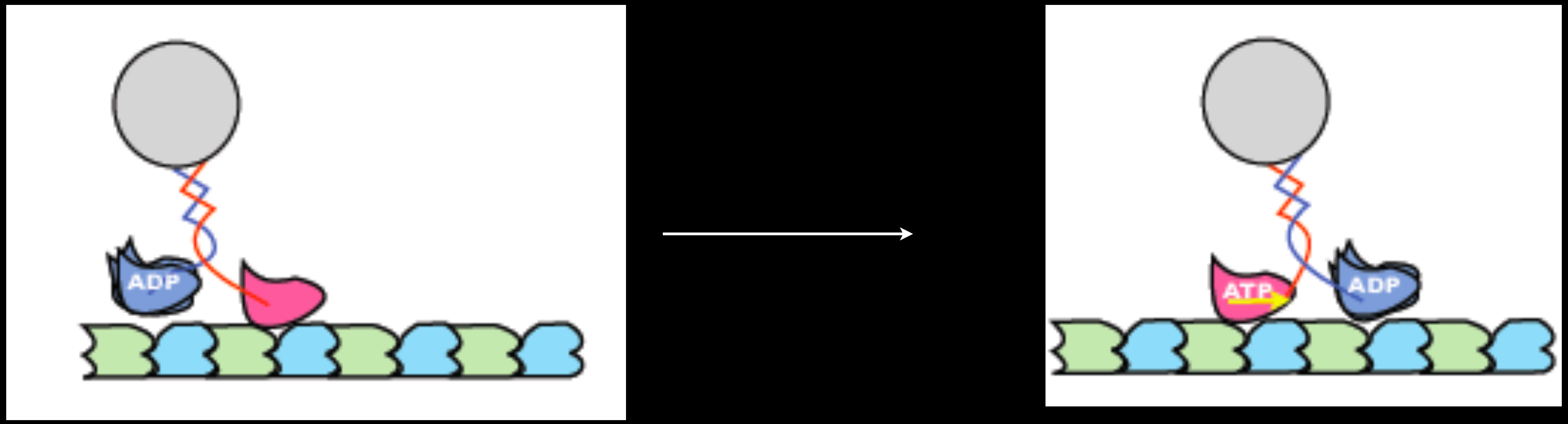
- Conversely, when kinesin is sped up by an assisting force, is it going through its normal biochemical cycle or by some other pathway ?

- When stepping processively, does kinesin spend most of its time in a two-heads bound (2HB) state or a one-head bound (1HB) state?

- Is the head-neck linker docking model correct (and does it suffice to explain actual stepping)? Does kinesin undertake a conformational "power stroke", or something like it (and if so, how large is it)?

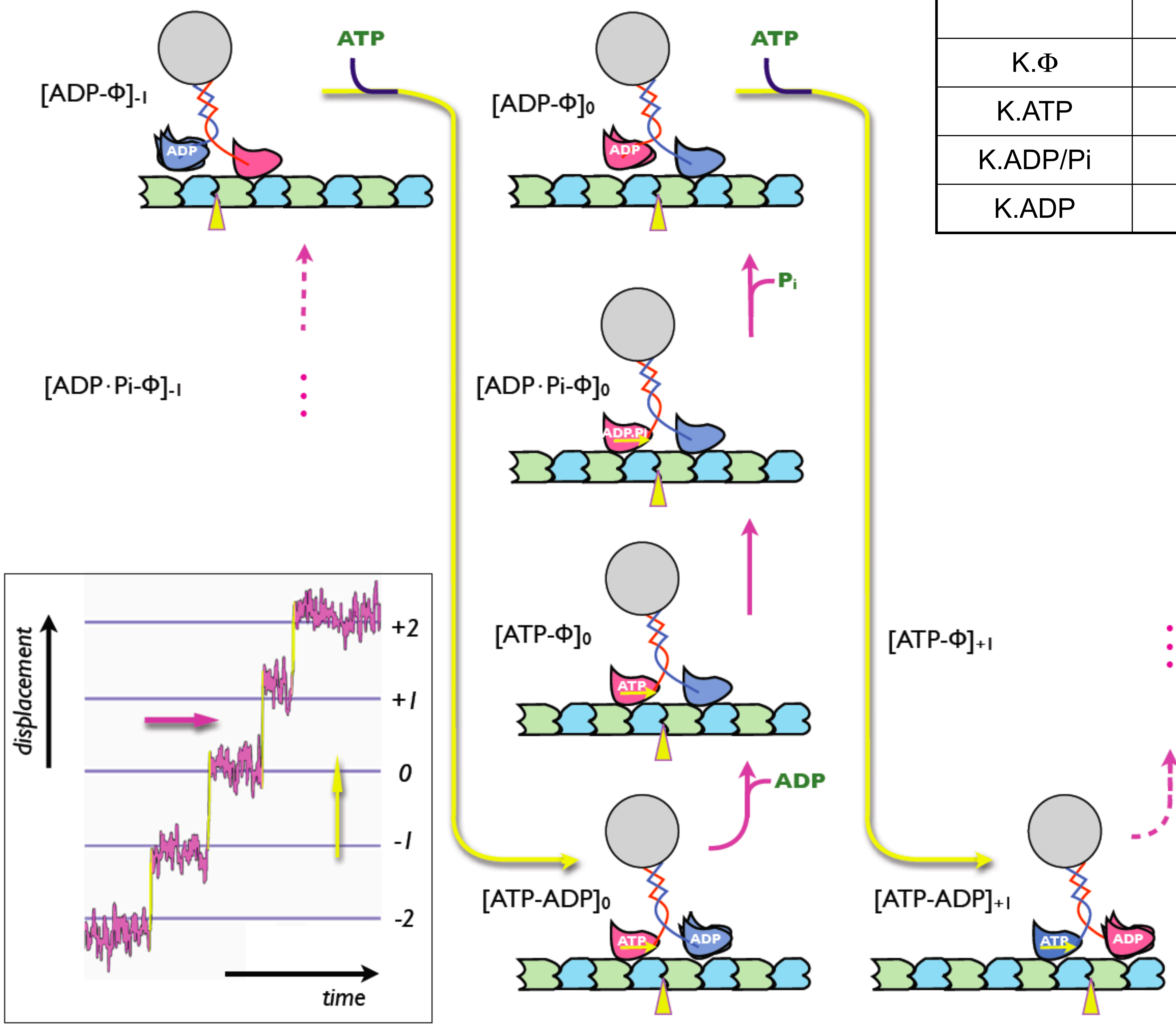
- How does kinesin manage to track parallel to a single protofilament of the microtubule?

Q: Where in the kinesin biochemical pathway is forward motion produced?



- Release of stored strain upon unbinding of the trailing head empowers an 8-nm advance of the entire molecules (Hancock & Howard)

- ATP binding induces the docking of the neck-linker on the leading head to produce motion of the partner head (Rice et al)



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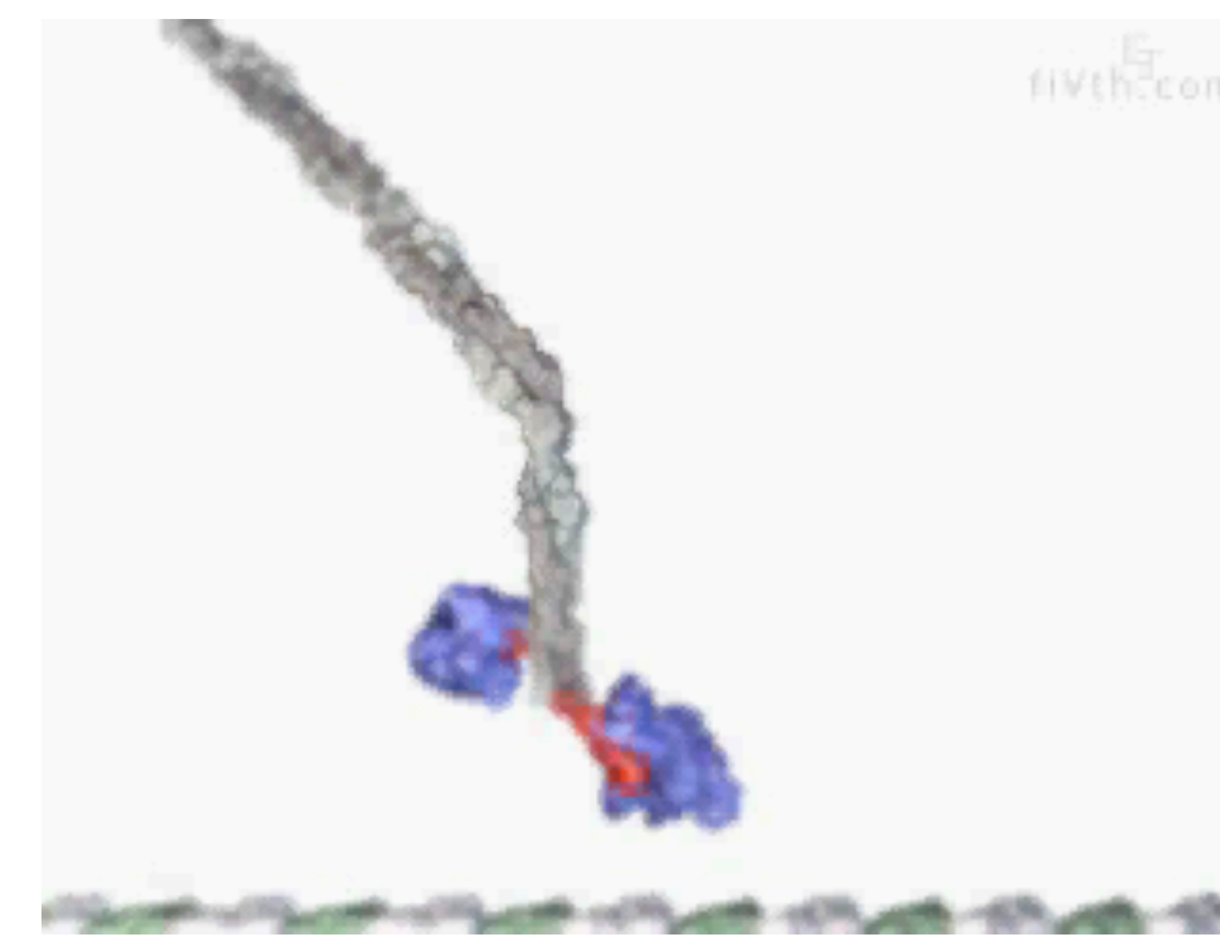
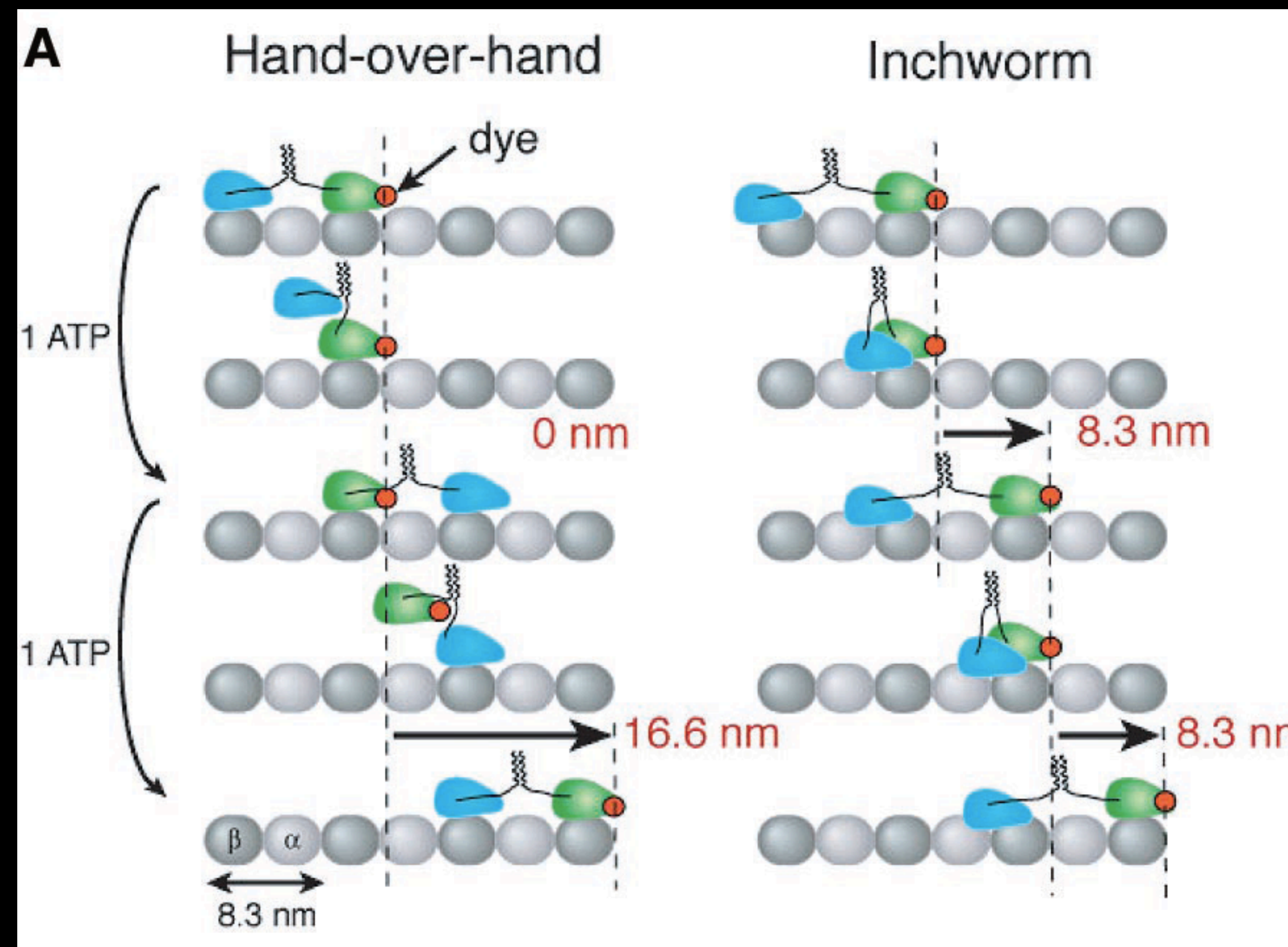
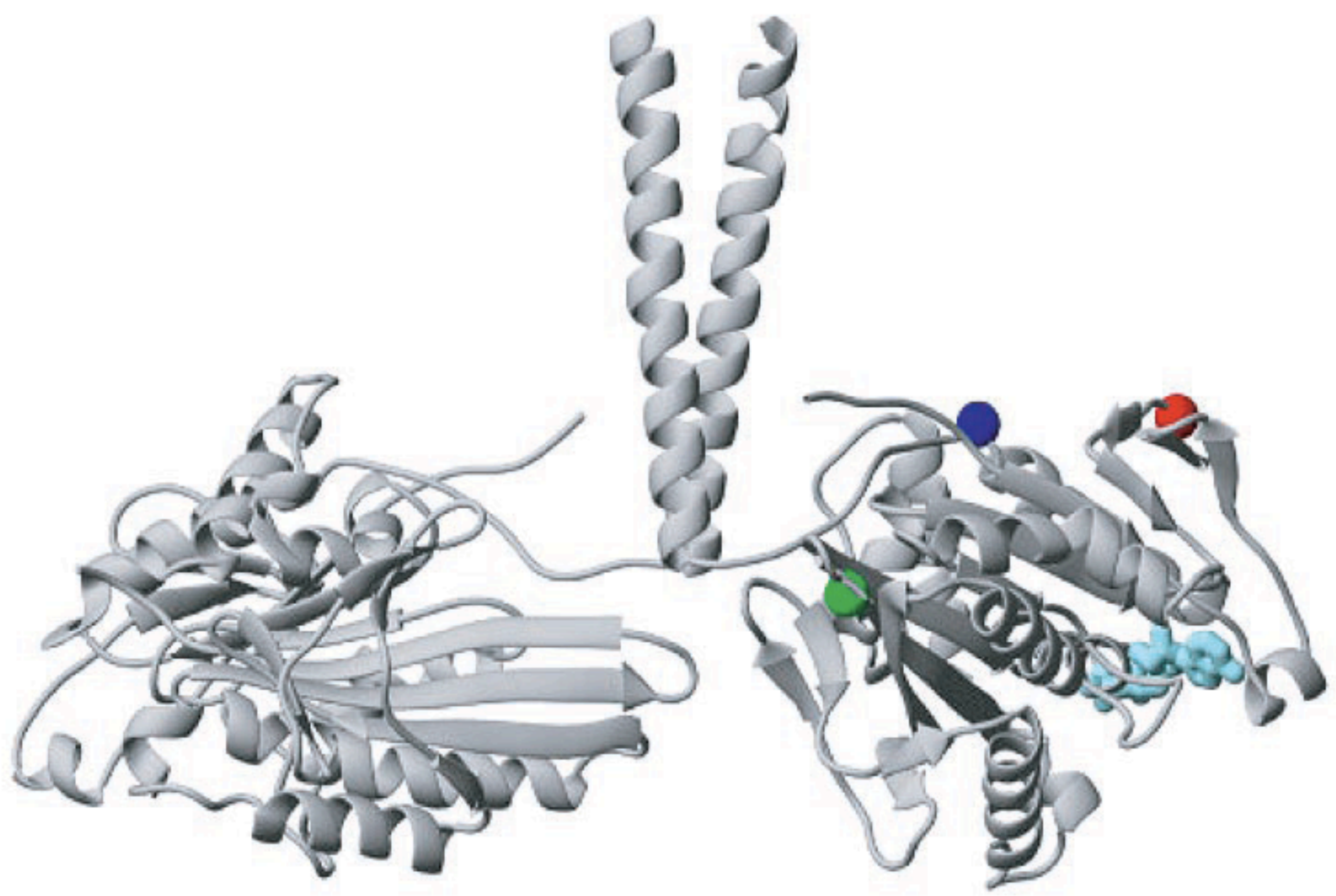
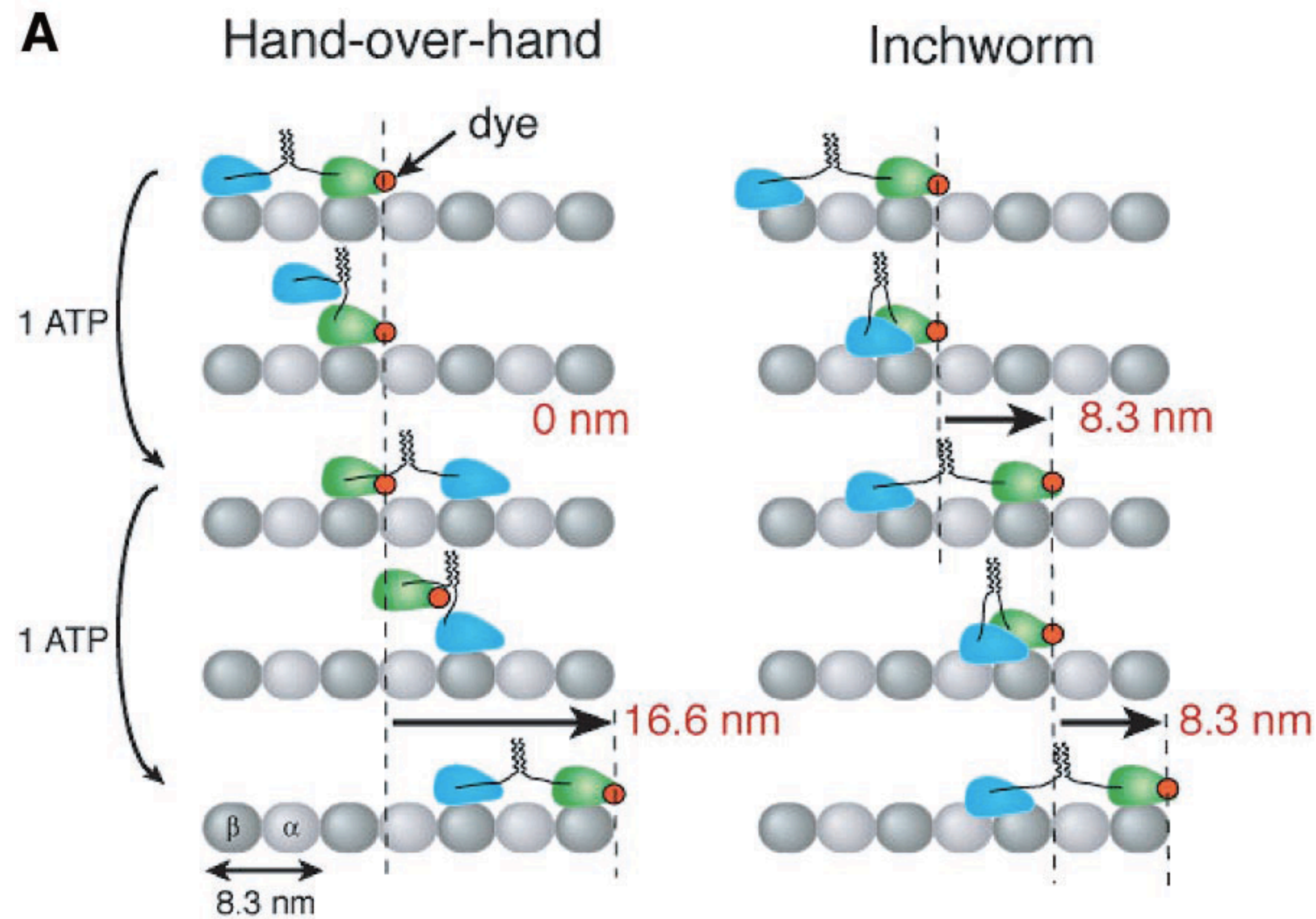


Q: What's the kinesin walking pattern, and what do we learn about its mechanics from this? (symmetric hand-over-hand, asymmetric hand-over-hand, inchworm)

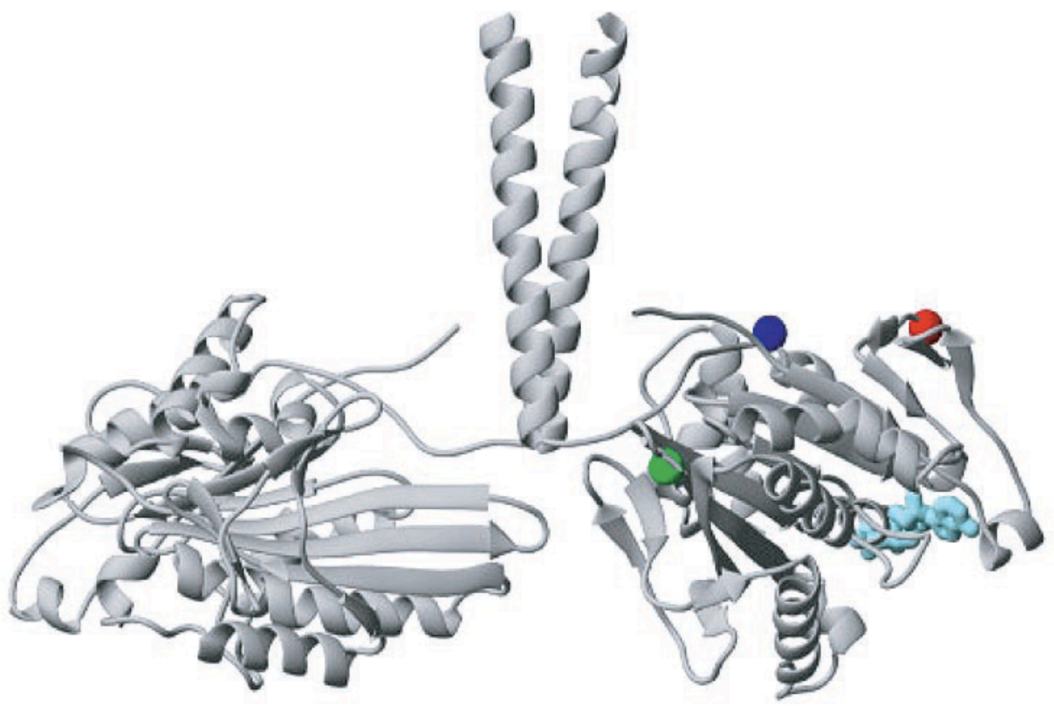


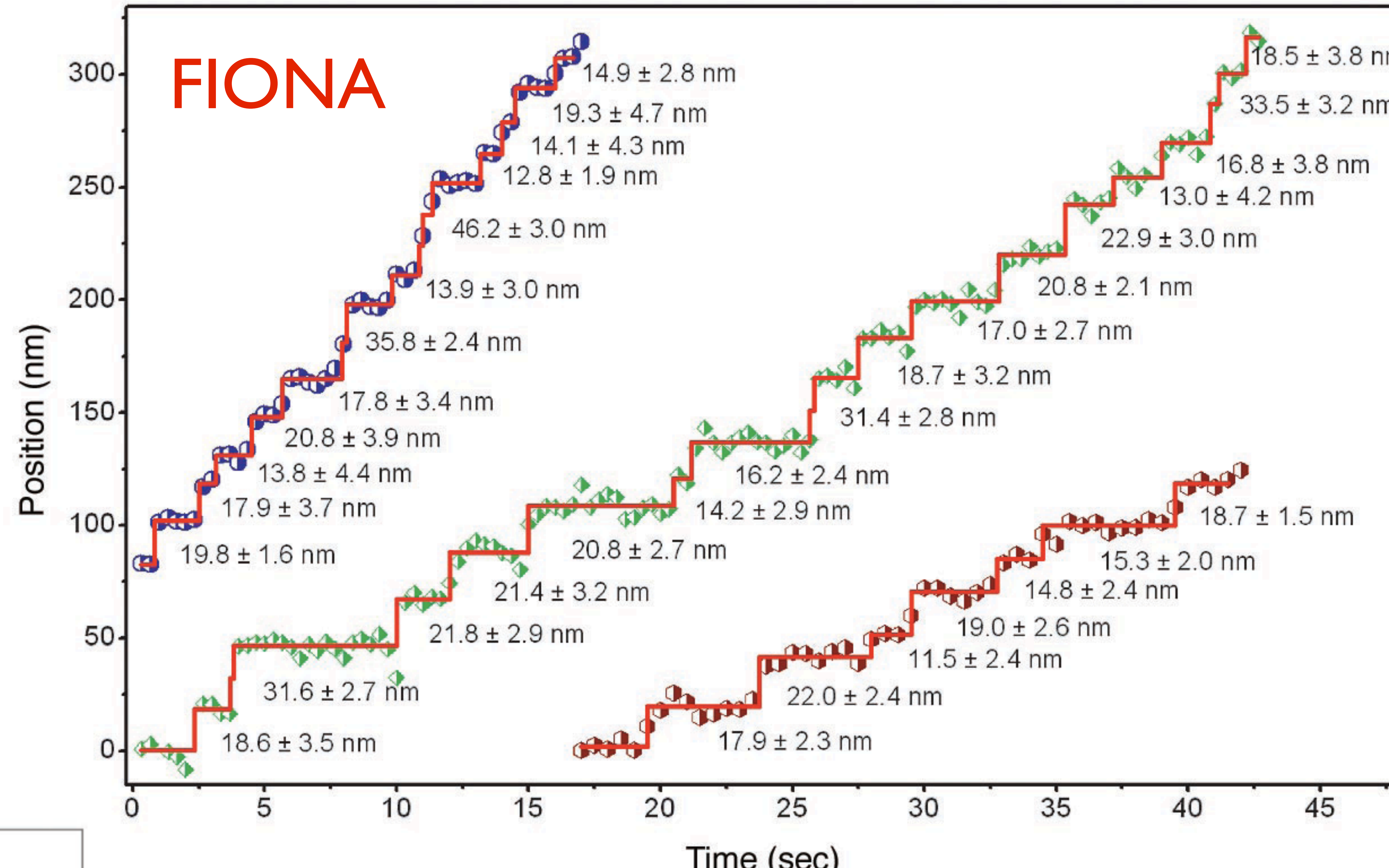


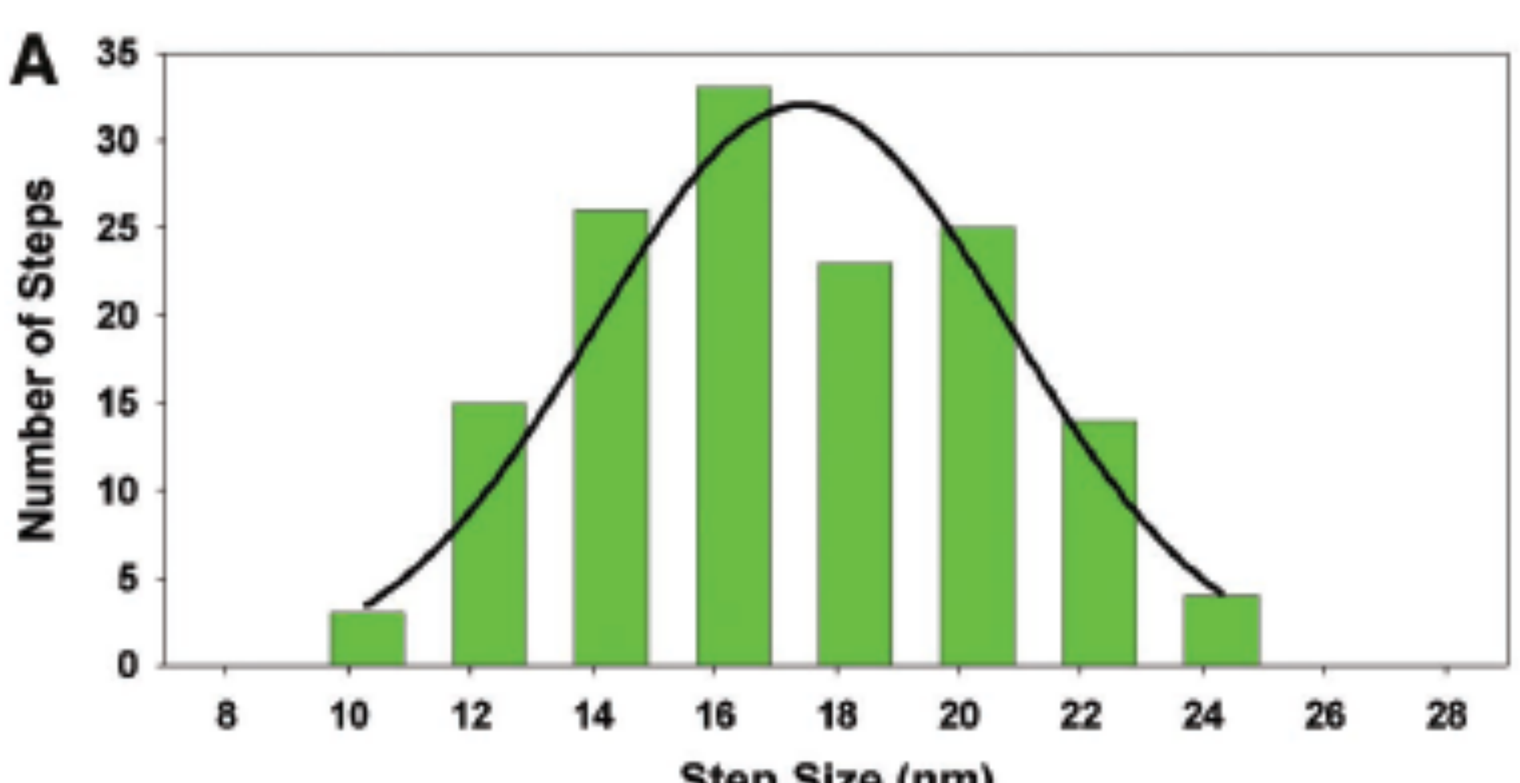


FIONA

Yildiz, Tomishige, Vale, Selvin Science (2004) 303: 676



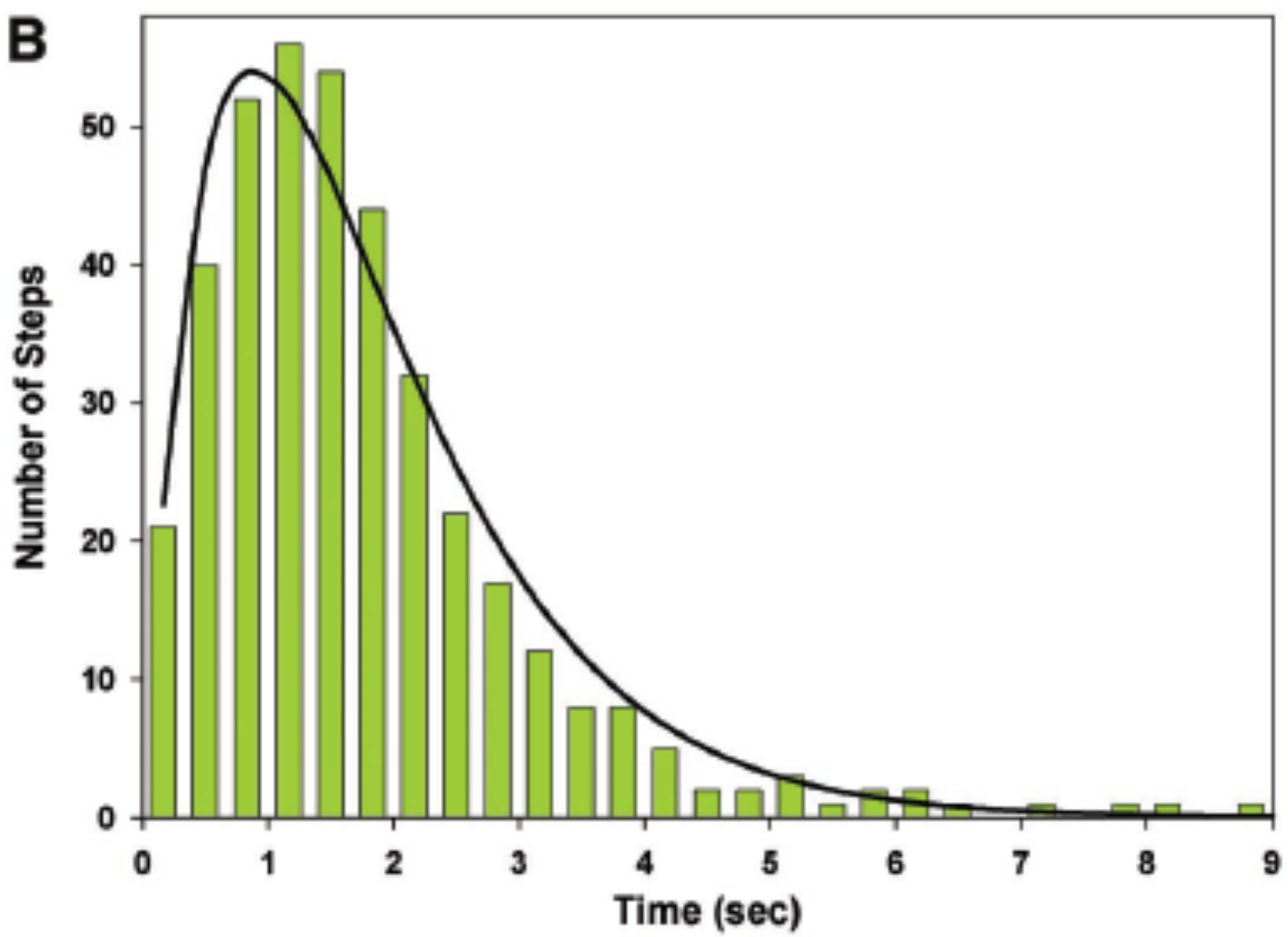




Yildiz, Tomishige, Vale, Selvin Science (2004) 303: 676

Dwell time distribution for the step of a single kinesin head : two poissonian steps with rate *k*

$$s \xrightarrow{k} p \xrightarrow{k} s \xrightarrow{k}$$



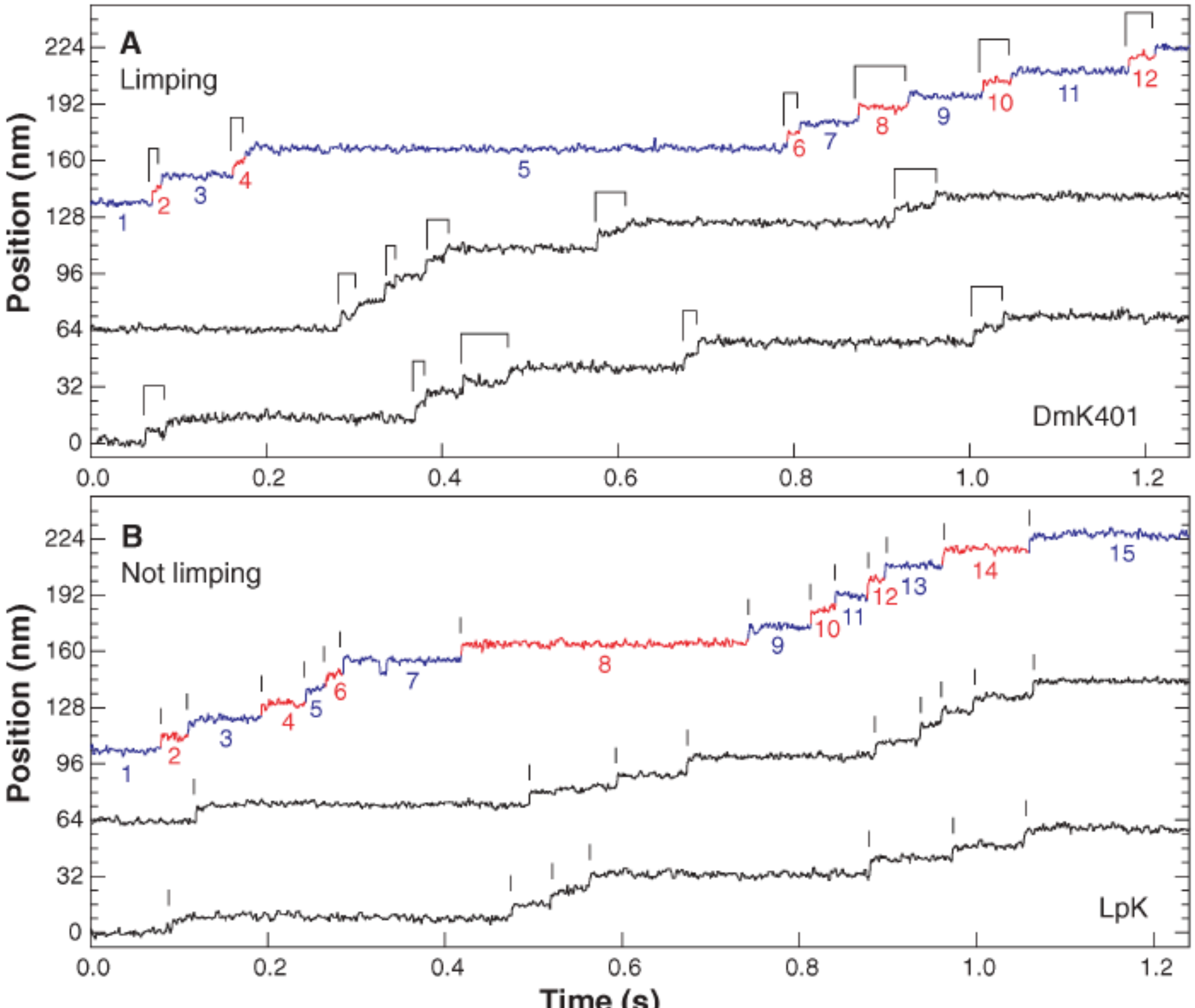
$$P(t) = \int_0^t d\tau P_1(\tau) P_2(t-\tau)$$

$$= \int_0^t d\tau k_1 e^{-k_1 \tau} k_2 e^{-k_2 (t-\tau)}$$

$$\lim_{k_1, k_2 \to k} P(t) = k^2 t e^{-kt}$$

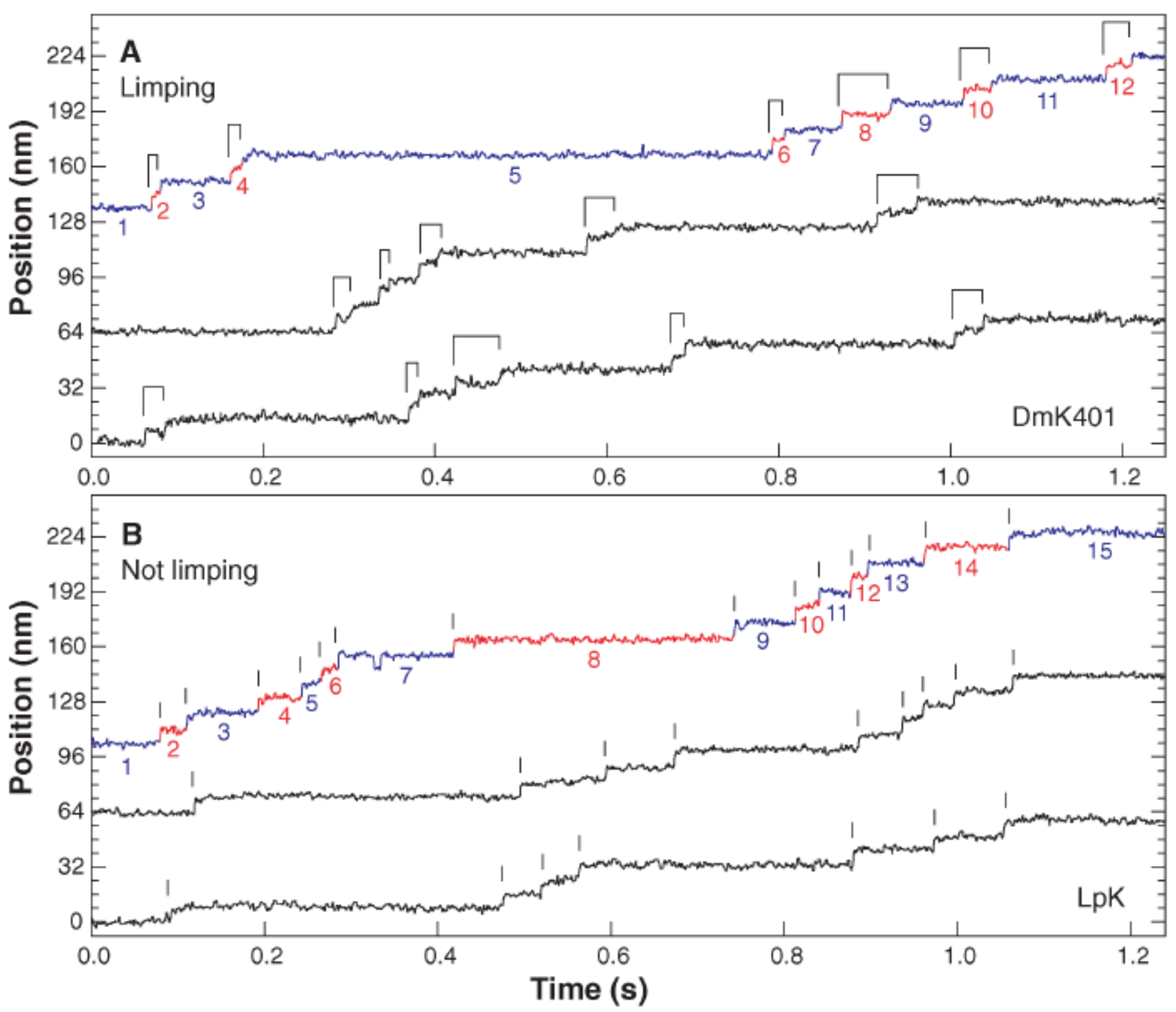
Yildiz, Tomishige, Vale, Selvin Science (2004) 303: 676

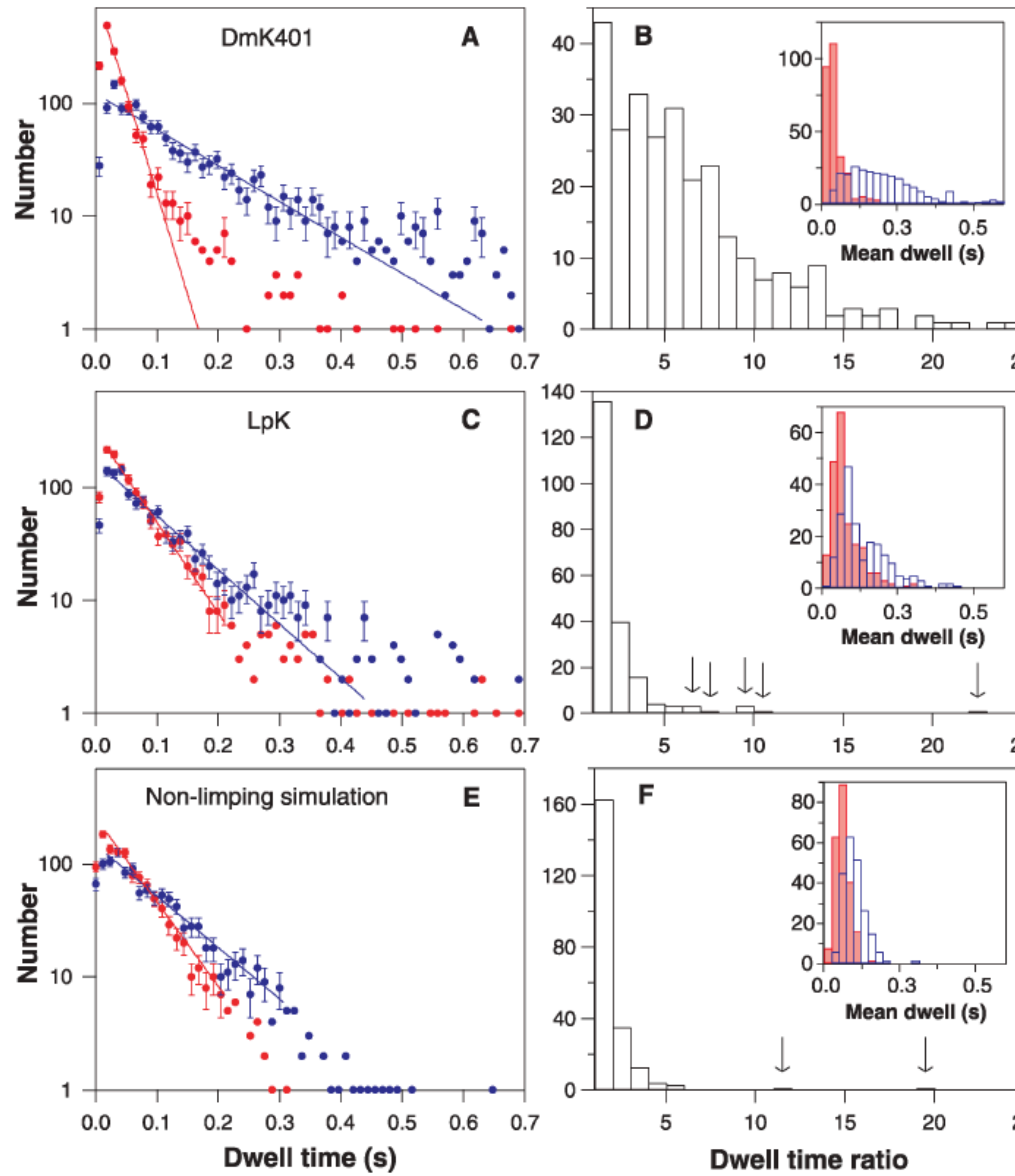
Symmetric HoH or Asymmetric HoH ?



Asbury et al. *Science* (2003) 302:2130

Symmetric HoH or Asymmetric HoH ?





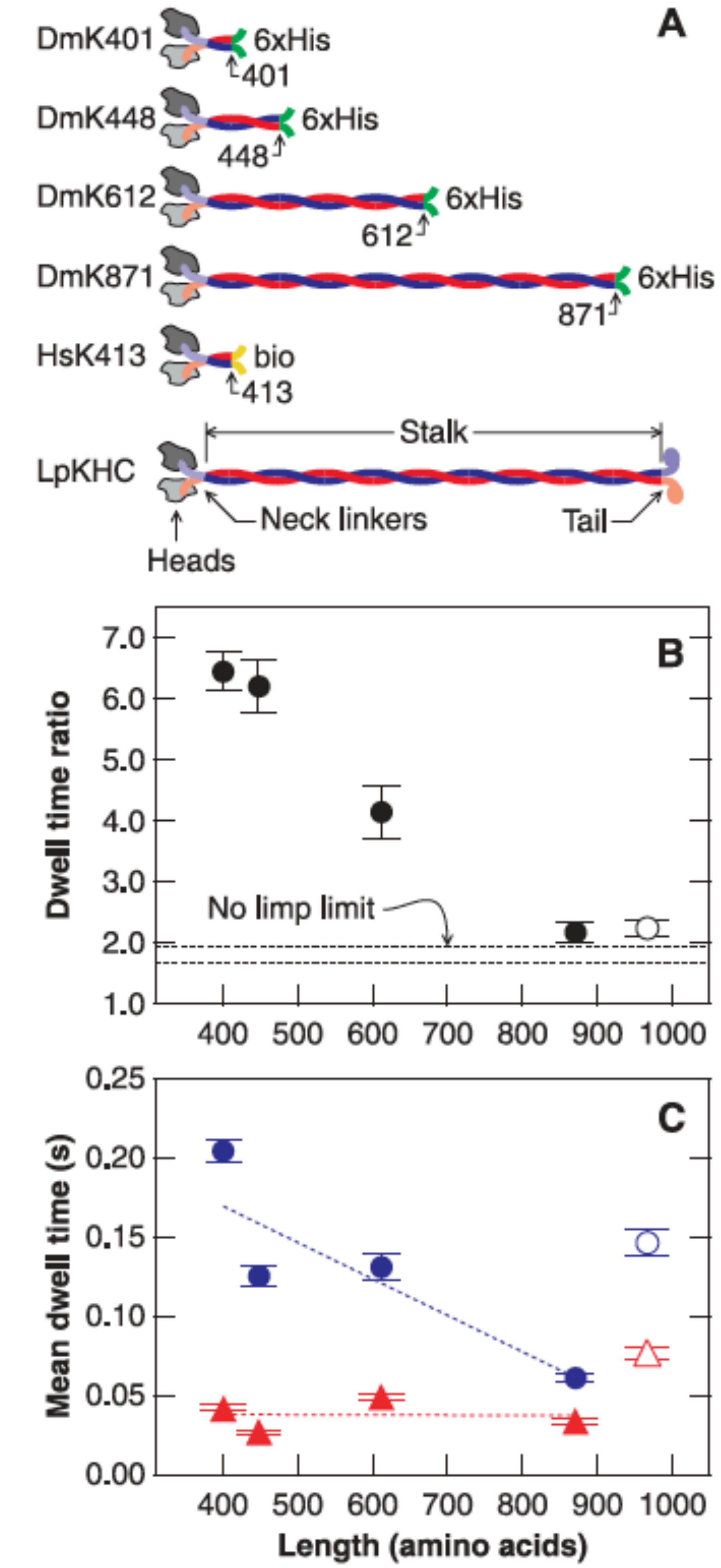
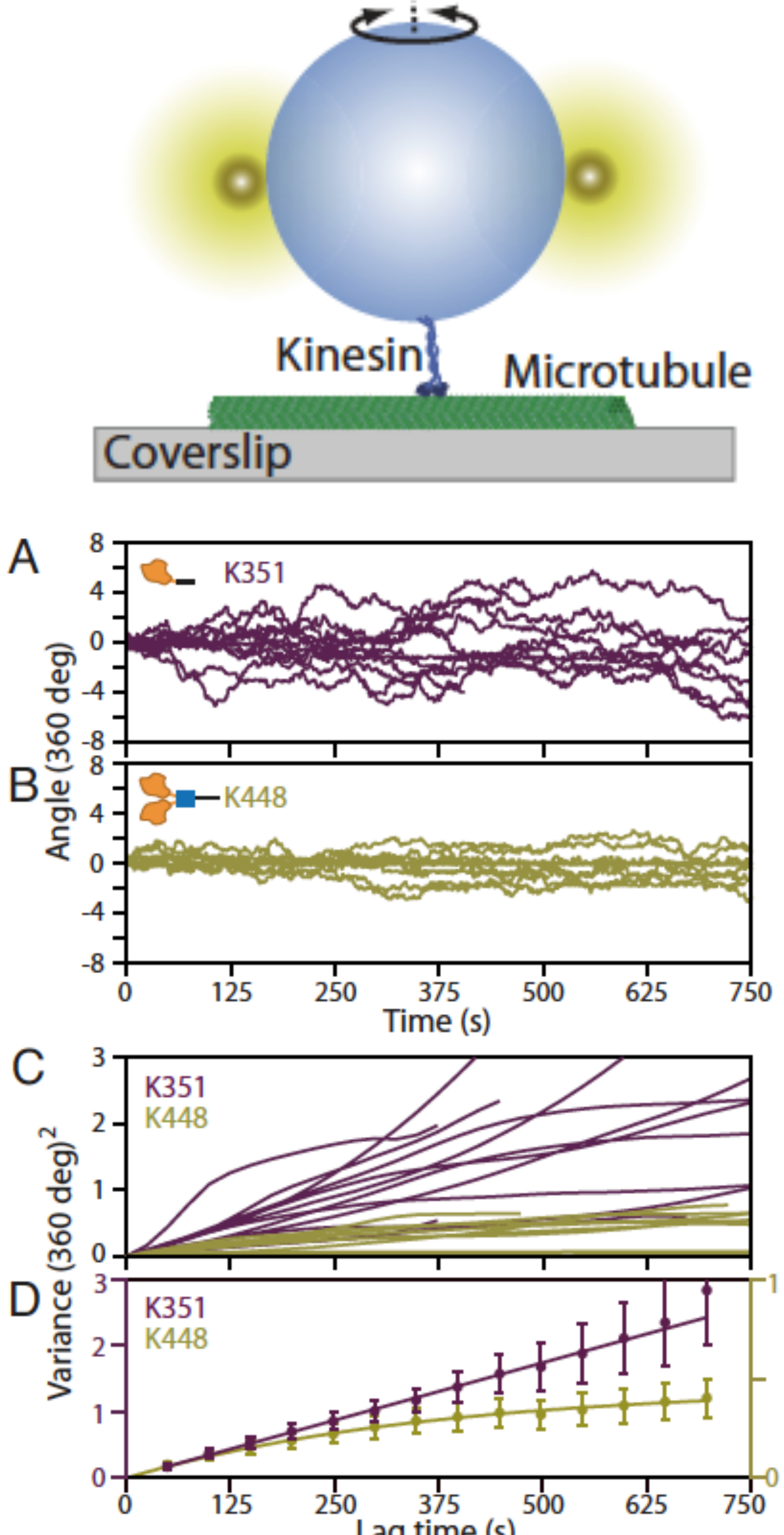
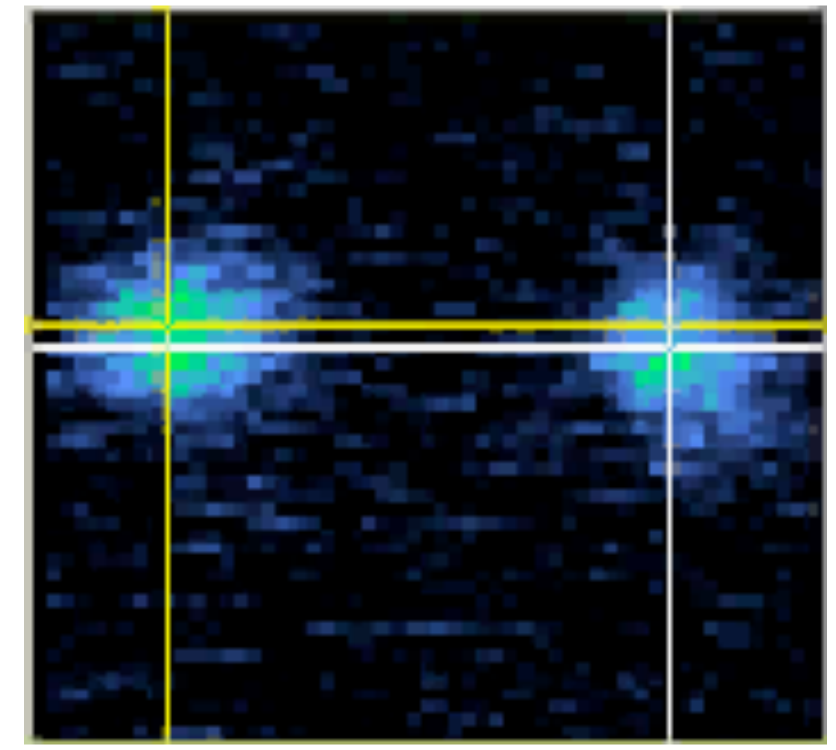


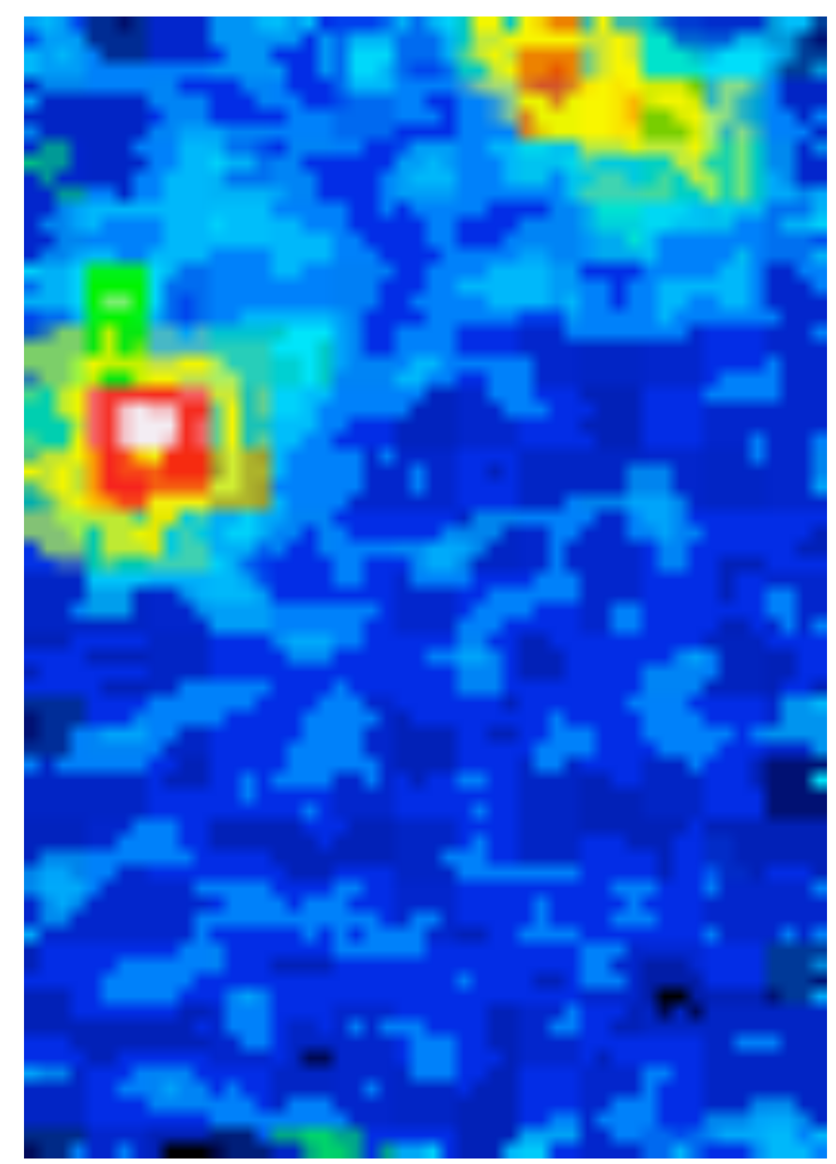
Fig. 3. The effect of construct length on kinesin

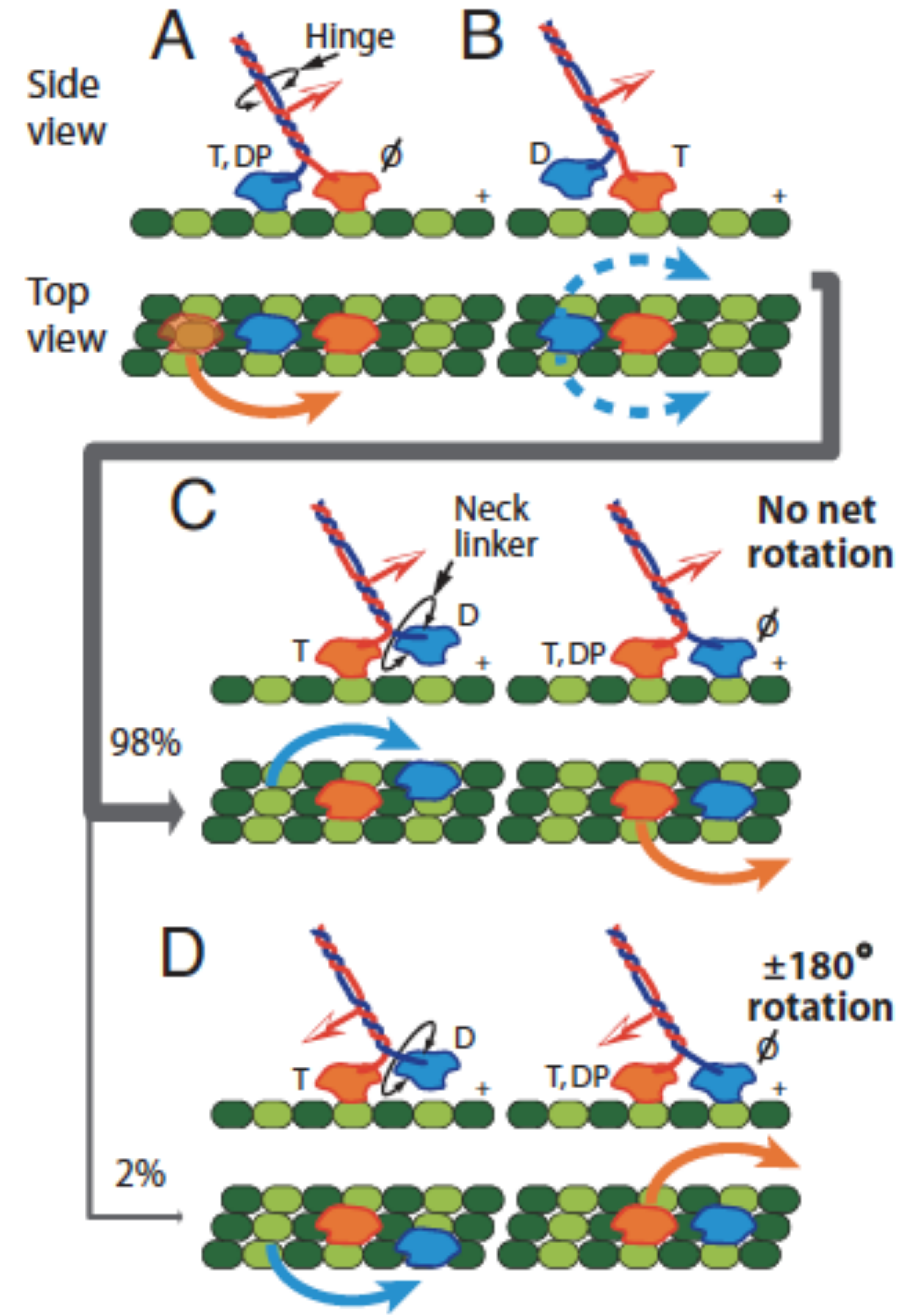
The longer the coiled-coil stalk, the less the kinesins limp

Asbury et al. *Science* (2003) 302:2130







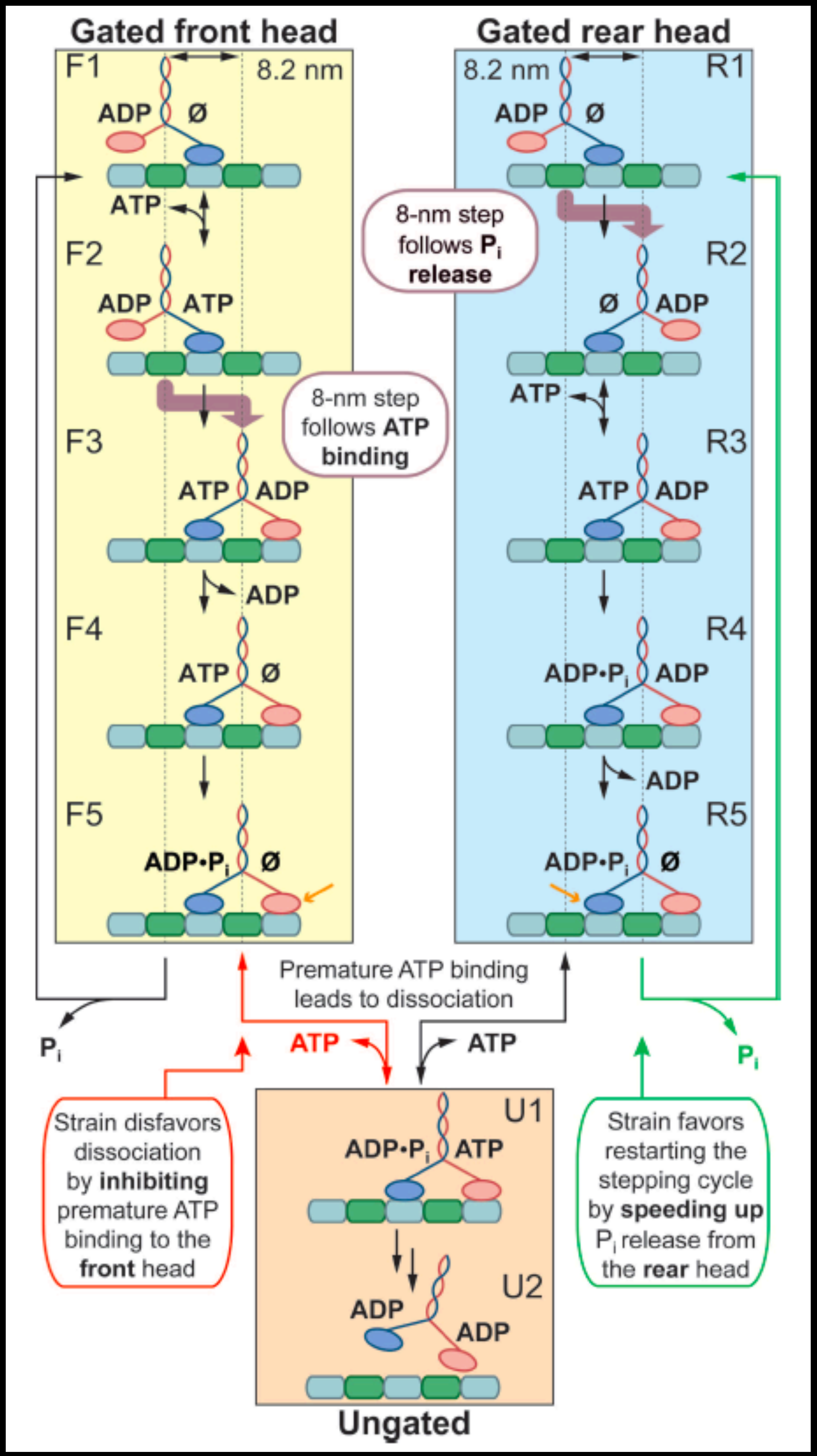


Block and coworkers PNAS (2009) 106:17007

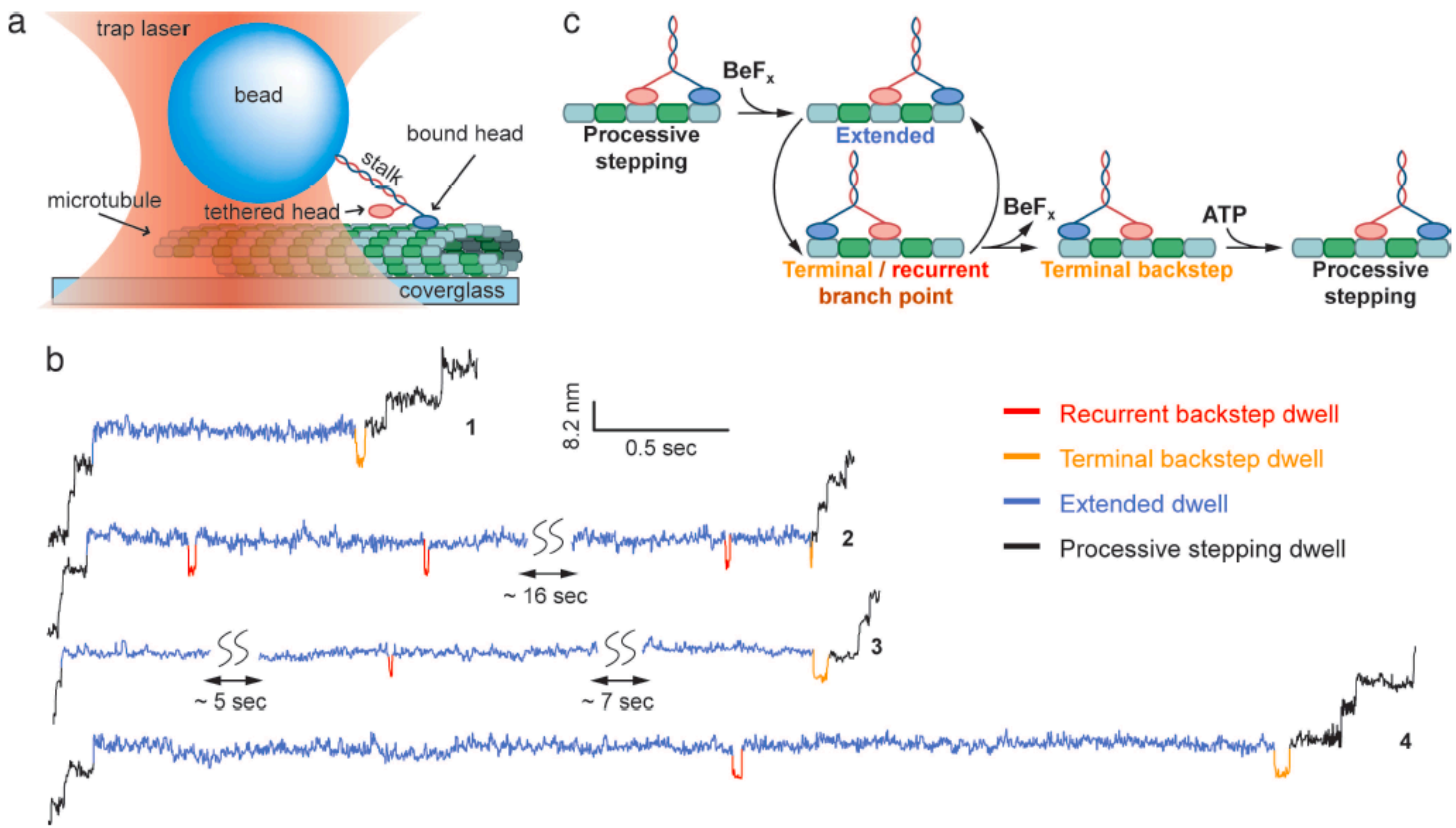
Q: How do the two kinesin heads manage to stay out of phase with one another during the stepping cycle (i.e., how are they “gated”)?

“Gated rear head” mechanism: *strain increases the detachment rate of the rear head from the MT.*

“Gated front head” mechanism: *ATP binding to the leading head is suppressed through internal strain.*

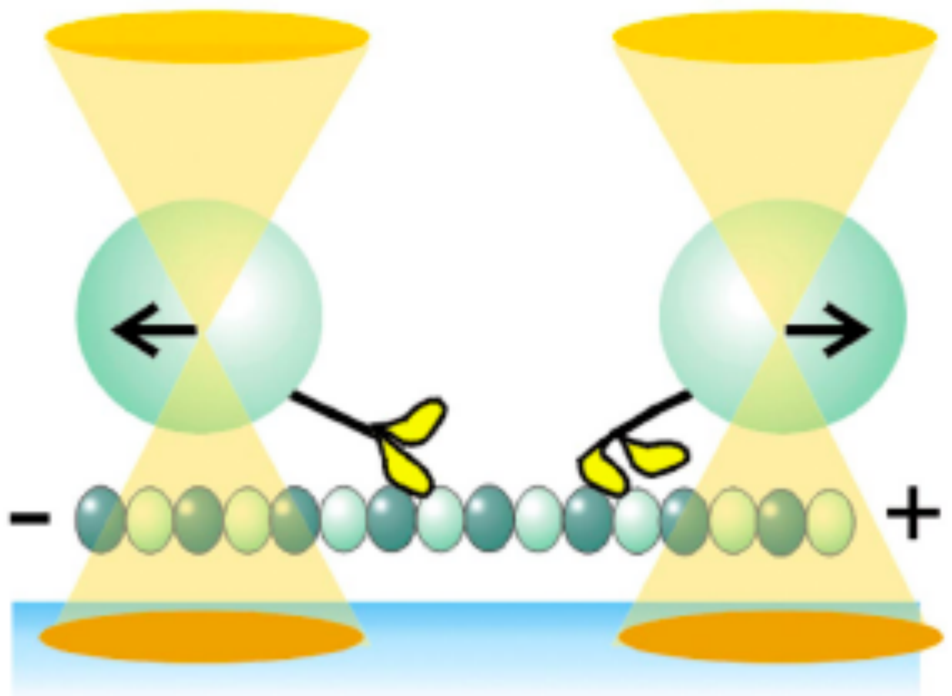


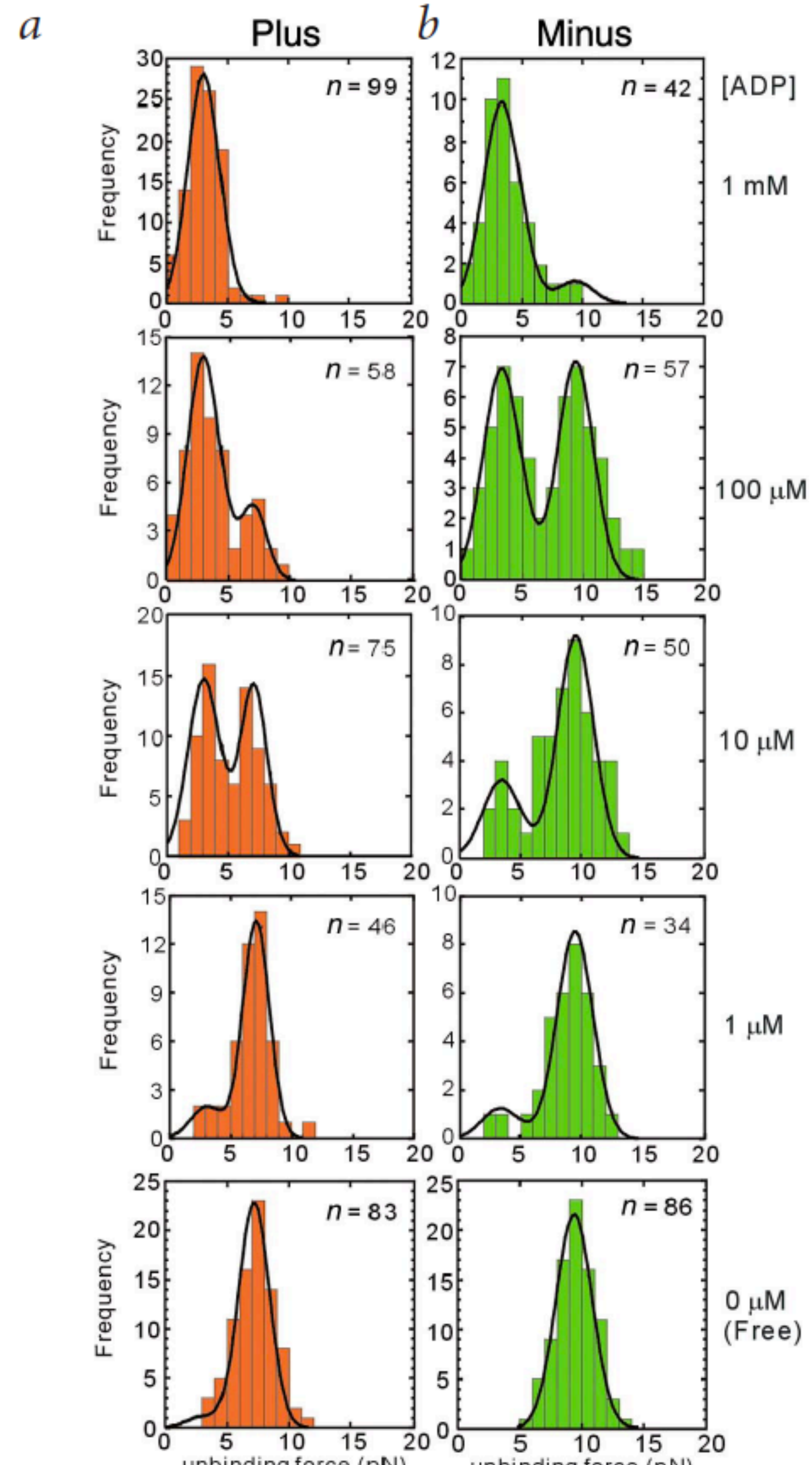
Guydosh & Block *PNAS* (2006) 103: 8054

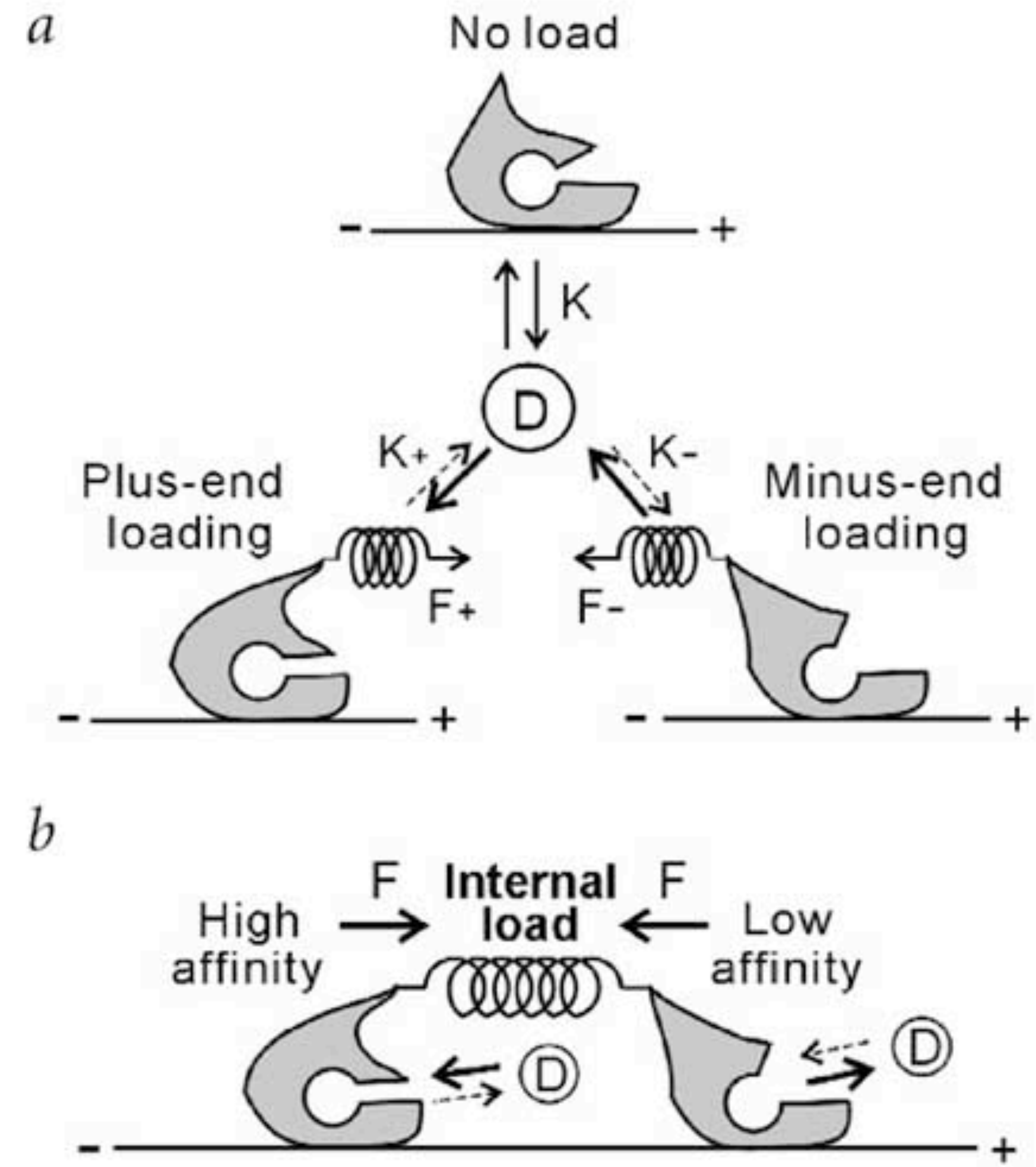


Addition of ADP·BeF$_x$ or AMP·PNP (ATP analog that strongly binds catalytic site) to kinesin causes extended dwell. Kinesin is rescued from this extended dwell only after a backstep →ATP analog dissociates when it is in the front head

Guydosh & Block *PNAS* (2006) 103: 8054

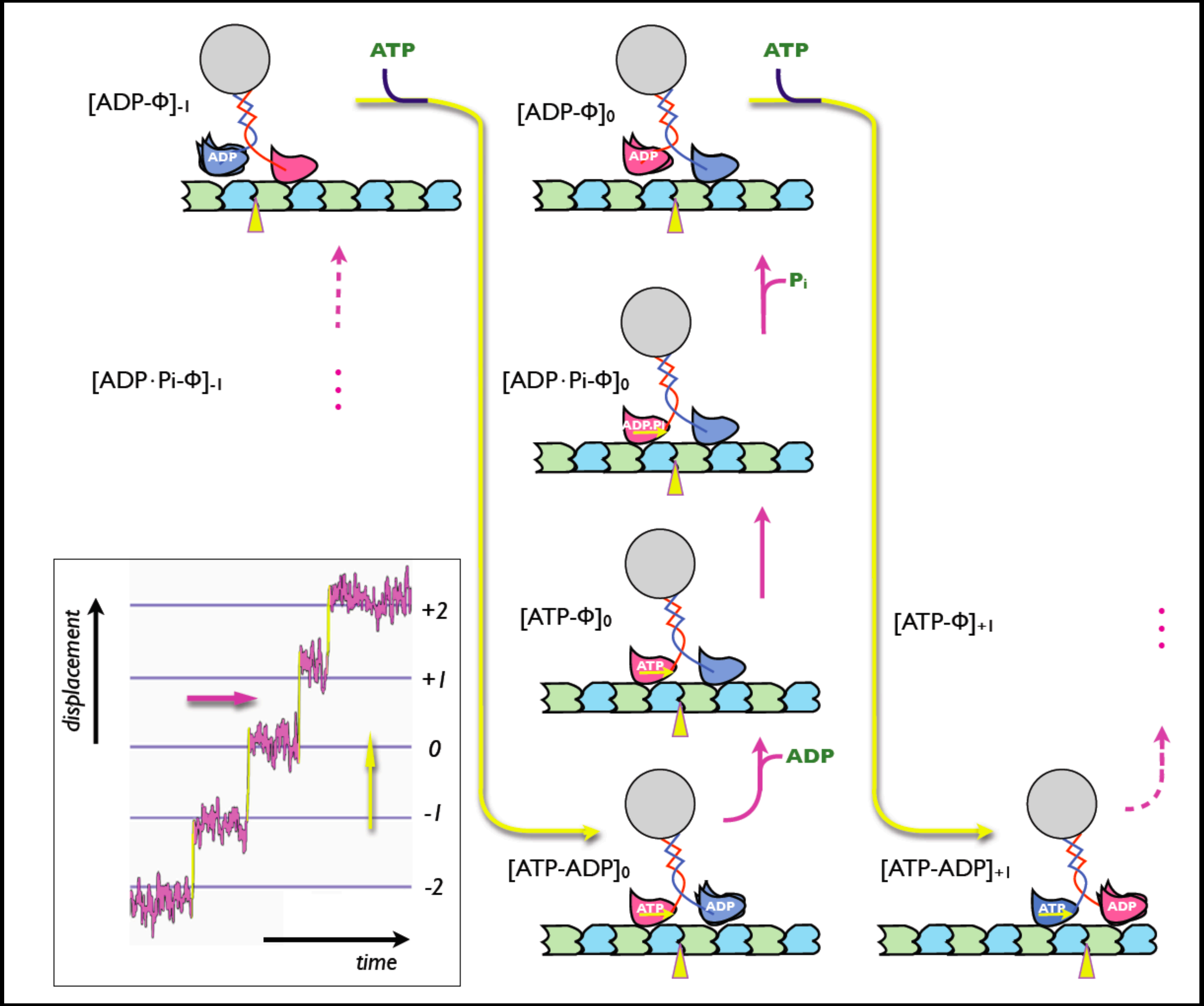


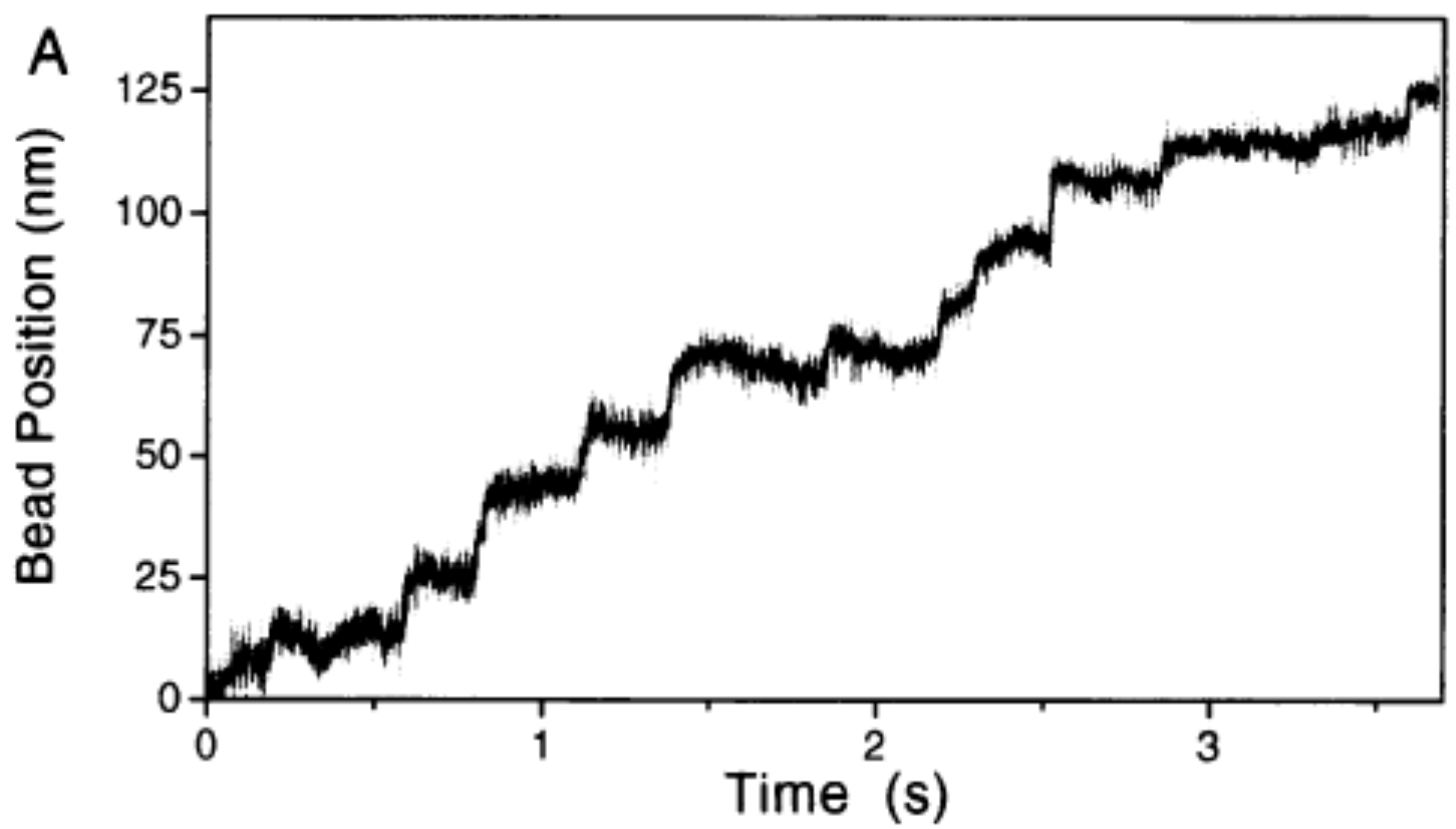


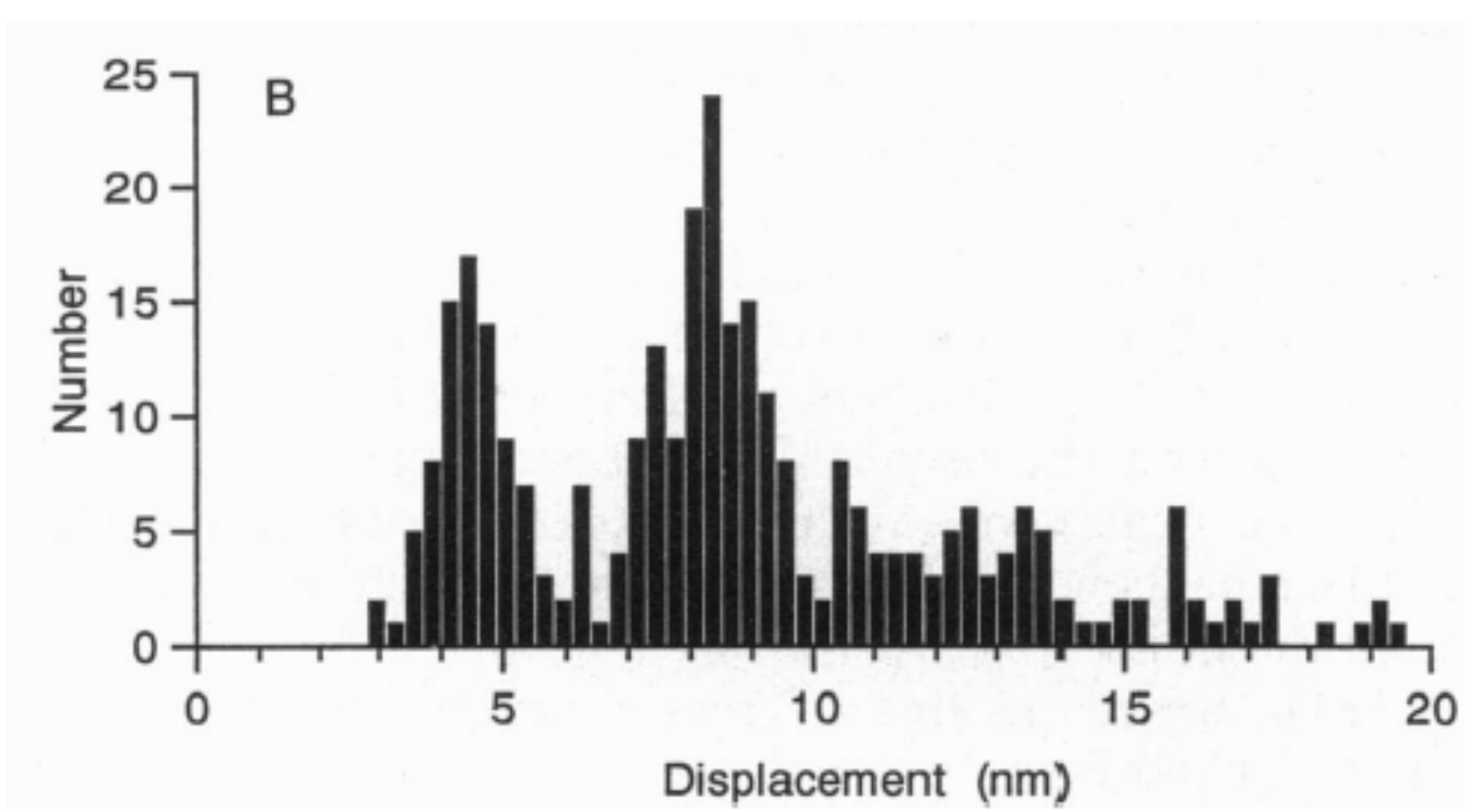


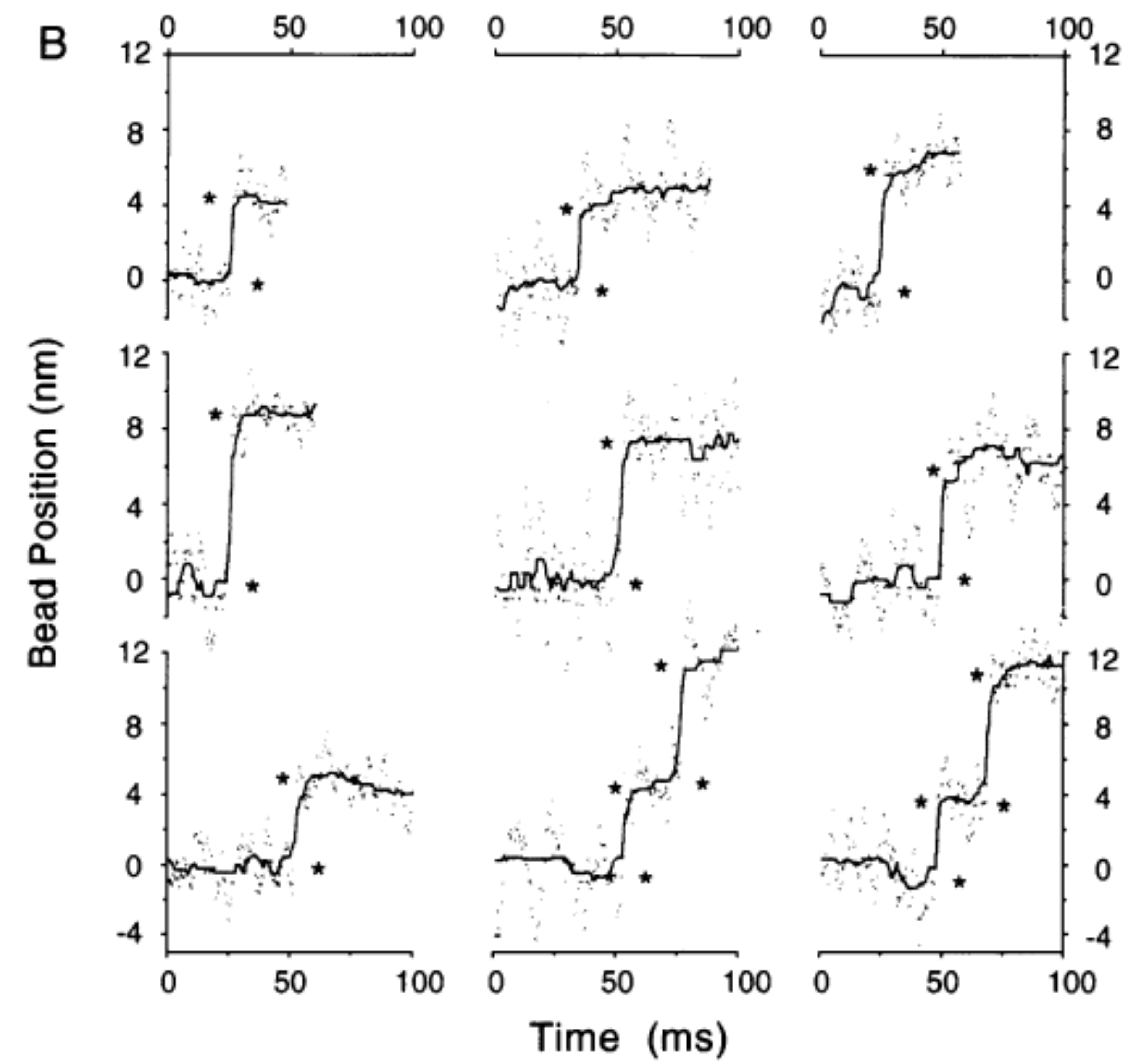
Uemura & Ishiwata *Nature Struct. Biol.* (2003) 10: 308

Q: Does kinesin take substeps? If so, over what time and distance scales?

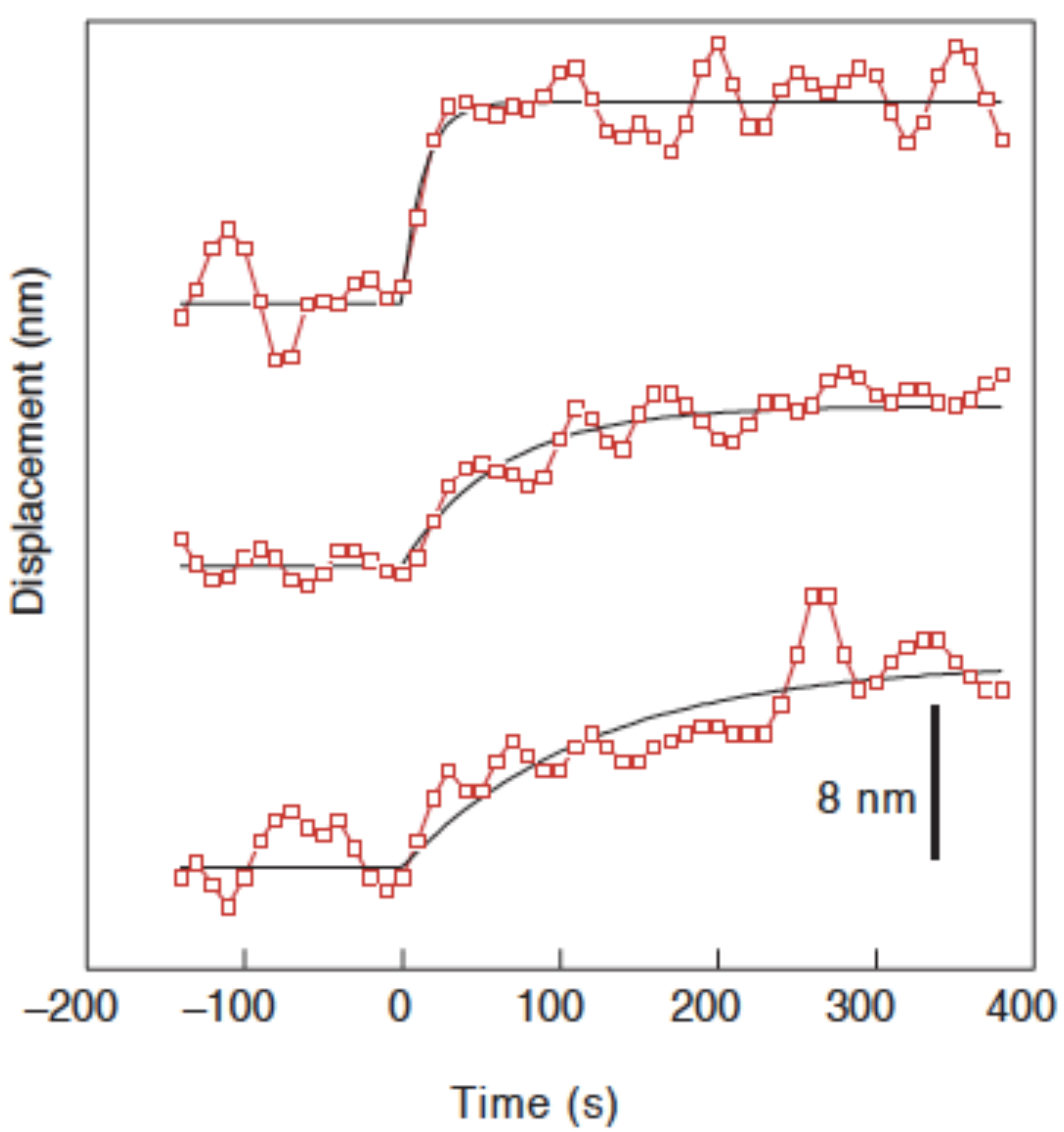


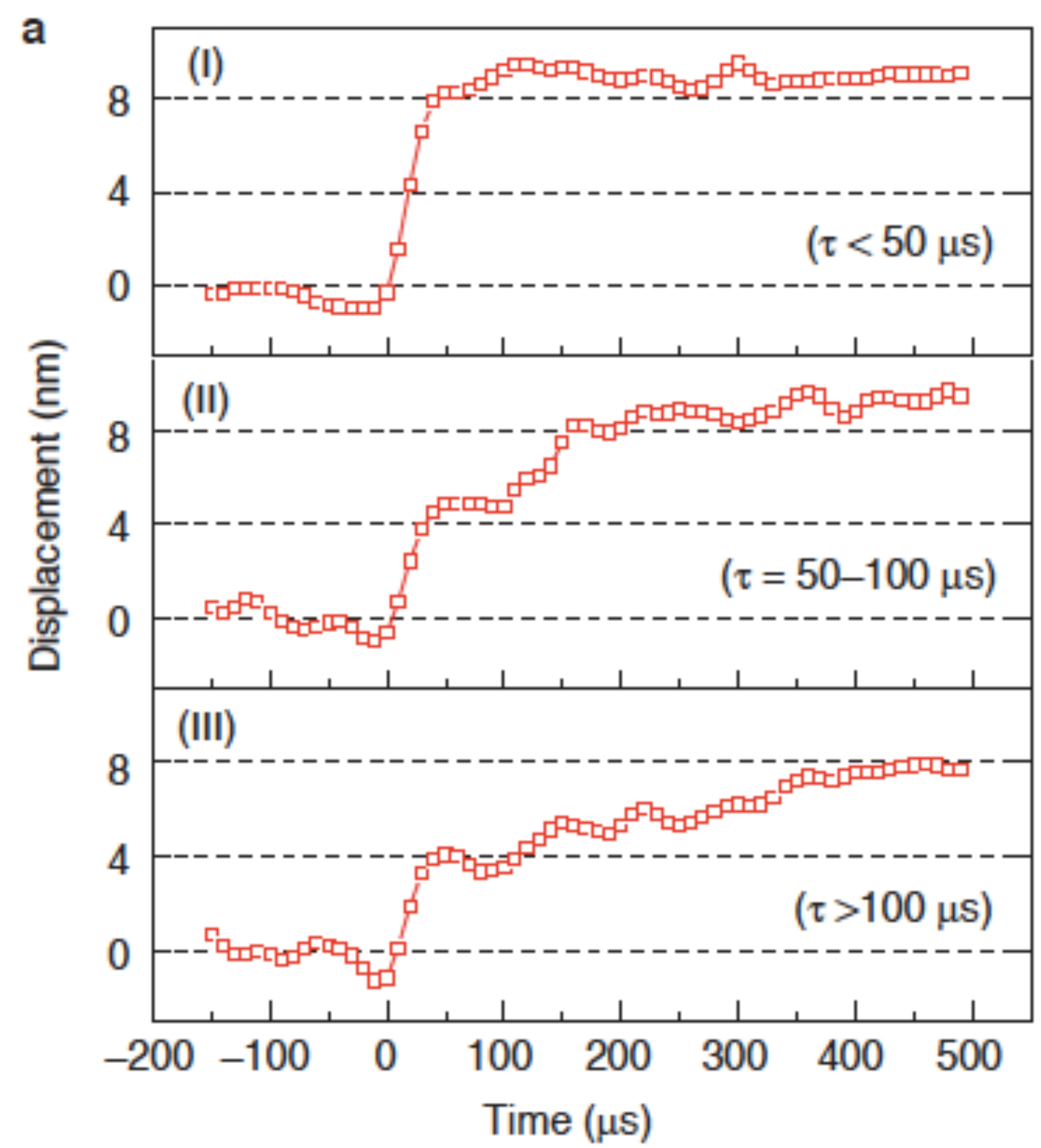


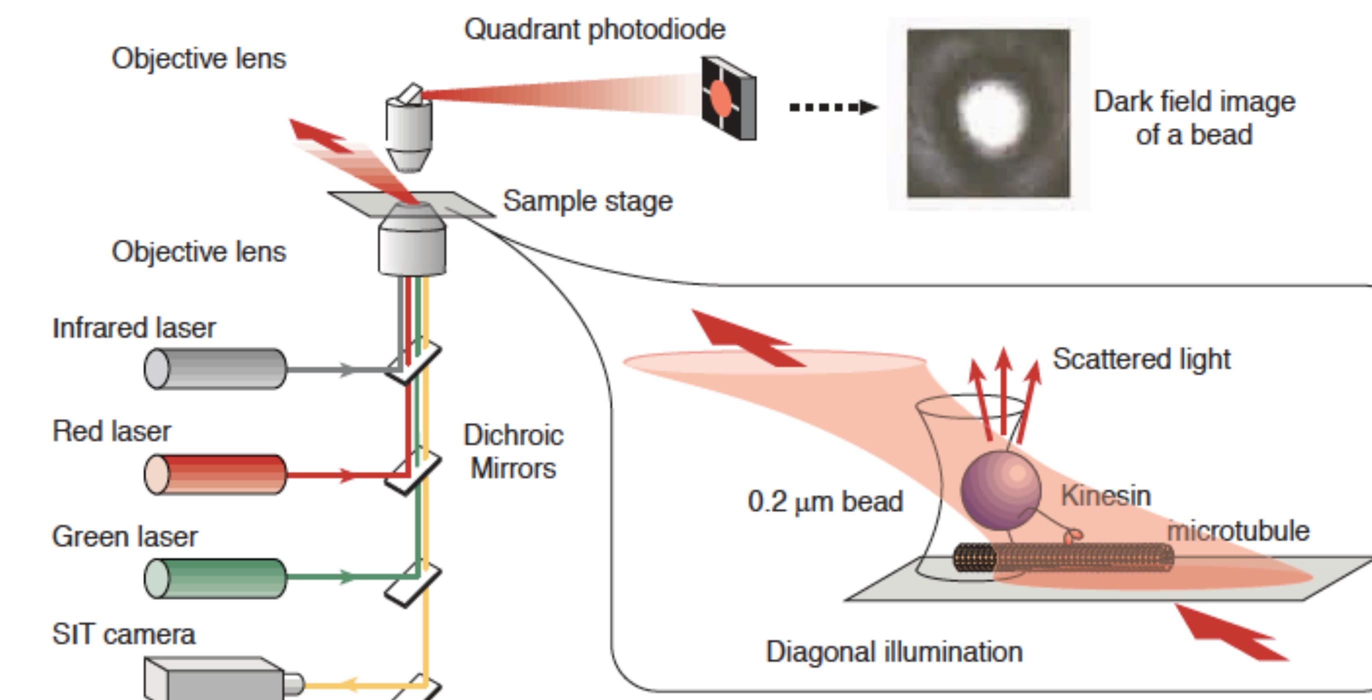


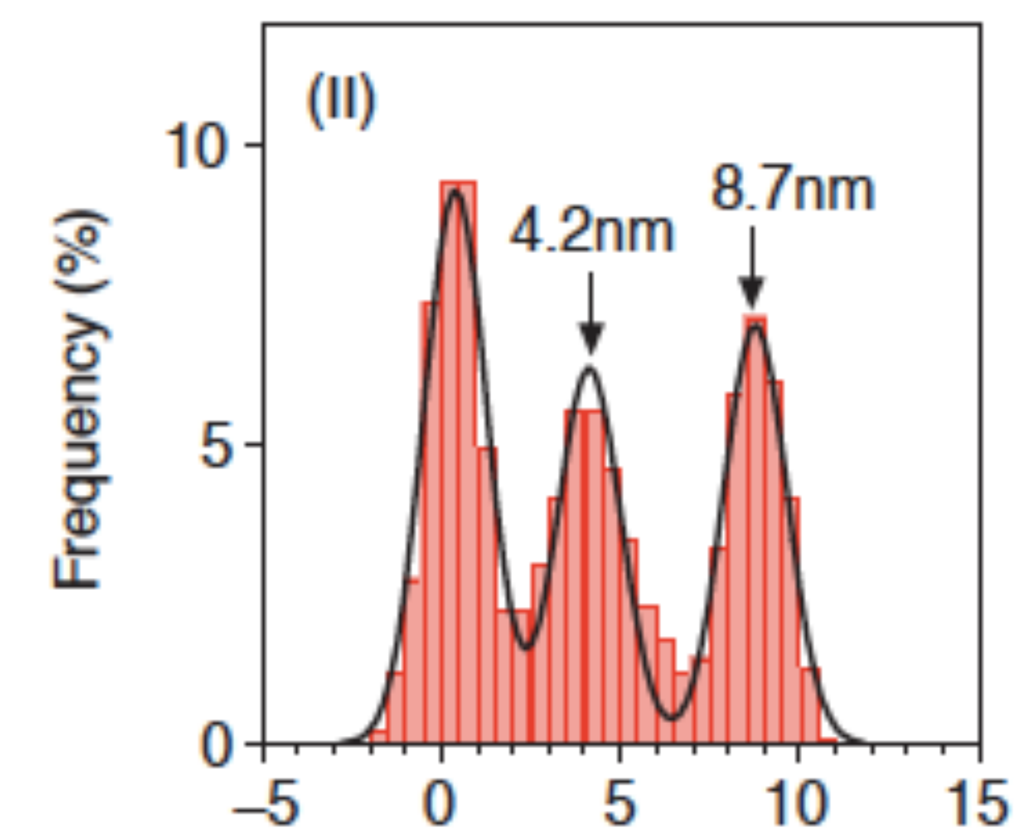


Coppin et al. PNAS (1996) 93:1913

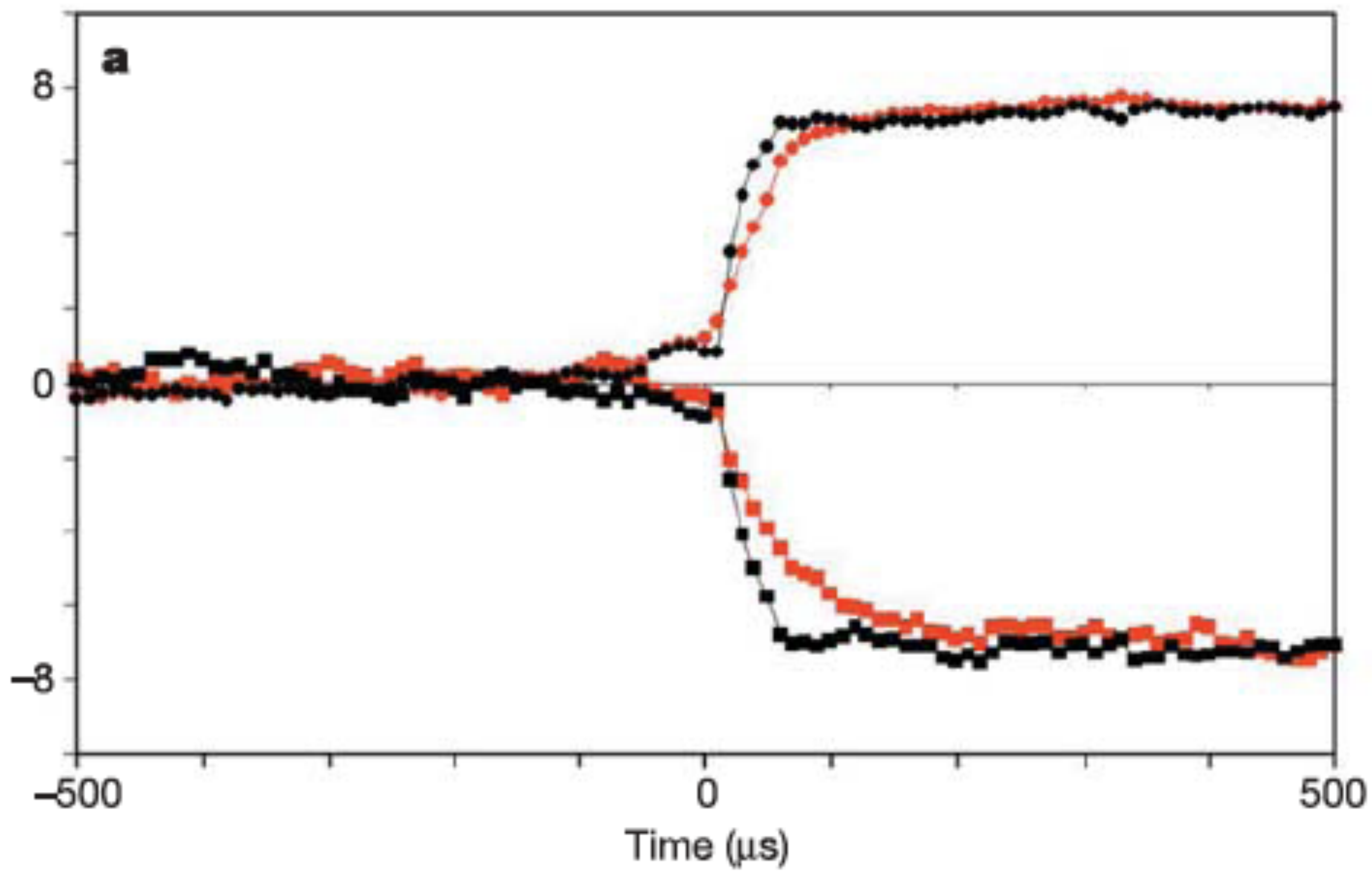








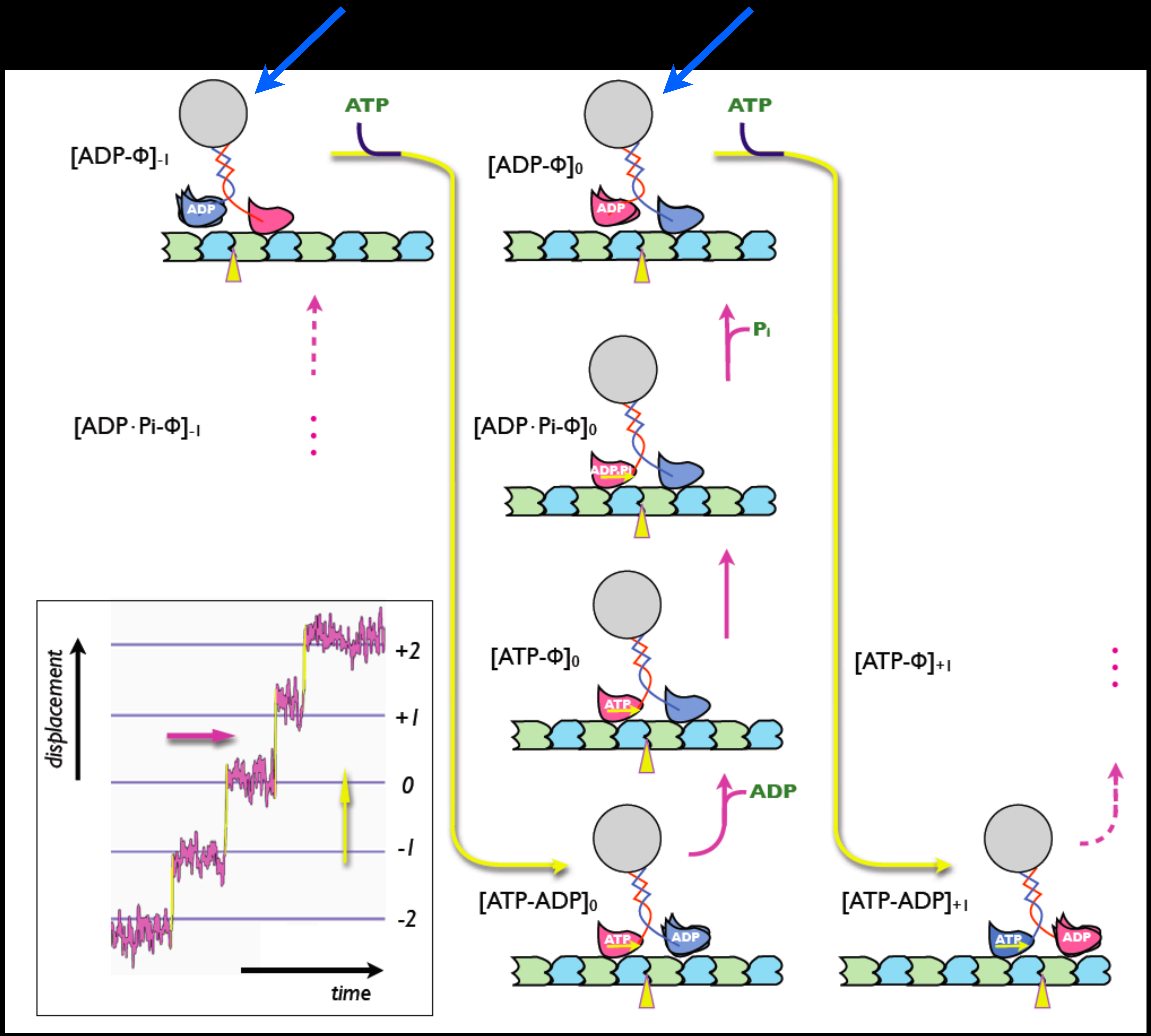
Yanagida and coworkers *Nature Cell Biol.* (2000) 3:425

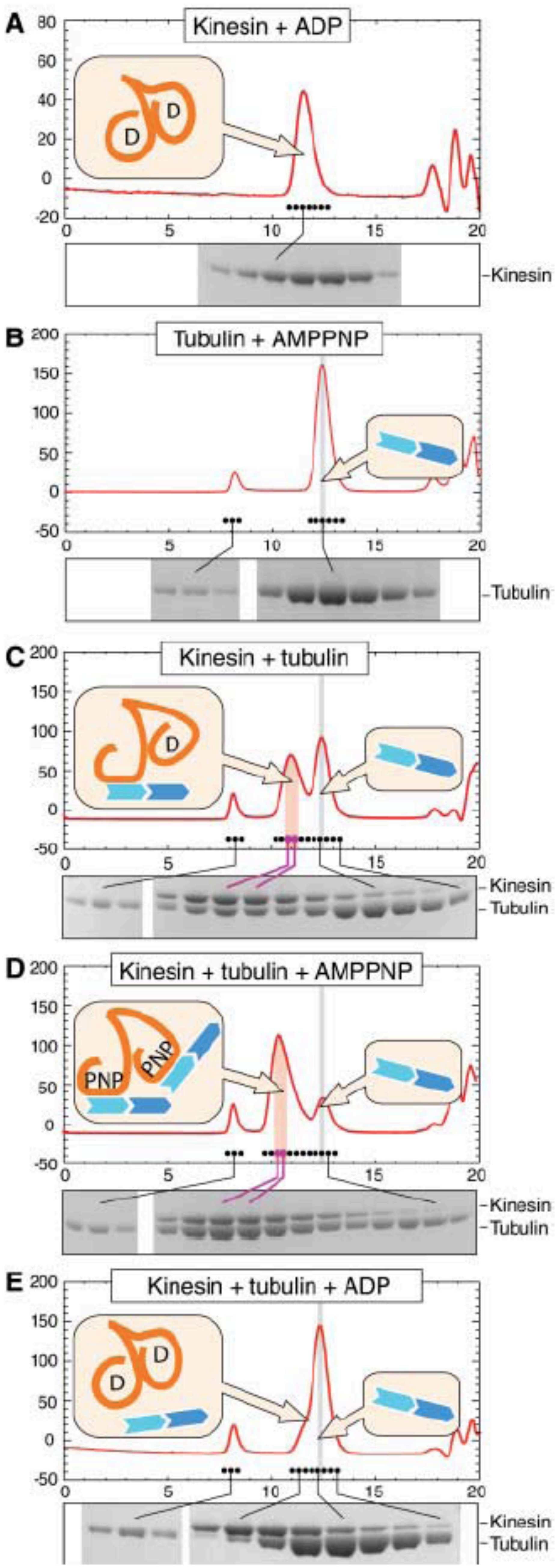


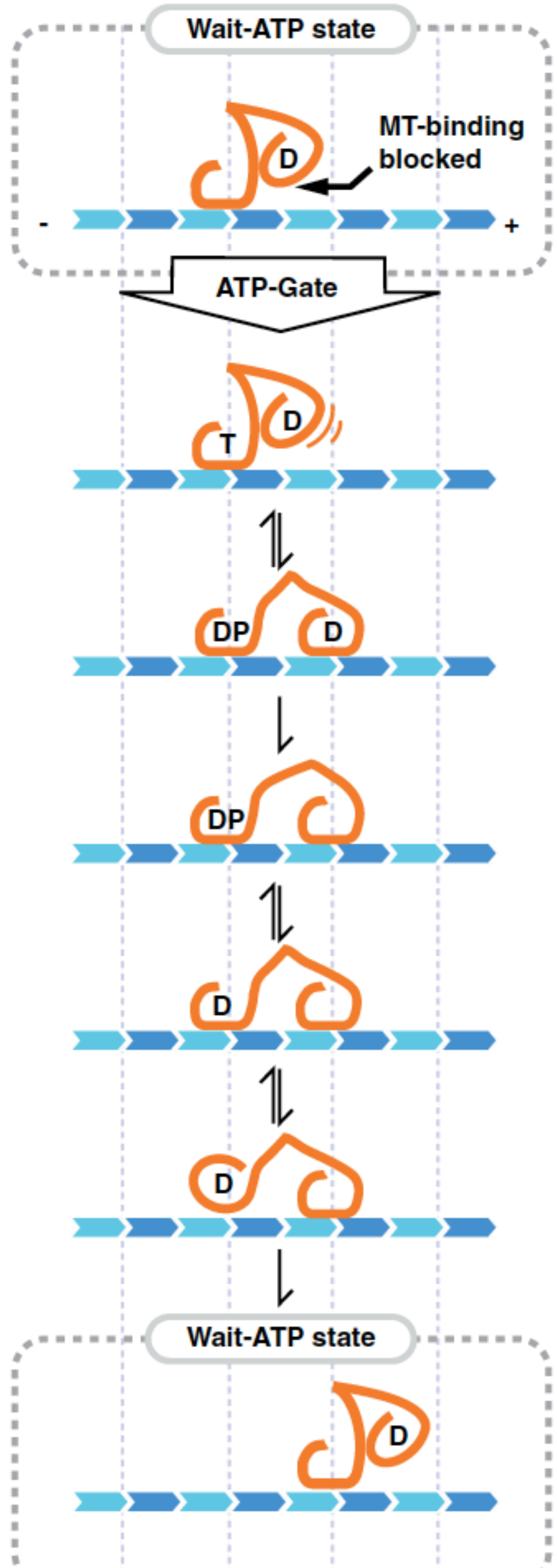
Carter & Cross *Nature* (2005)

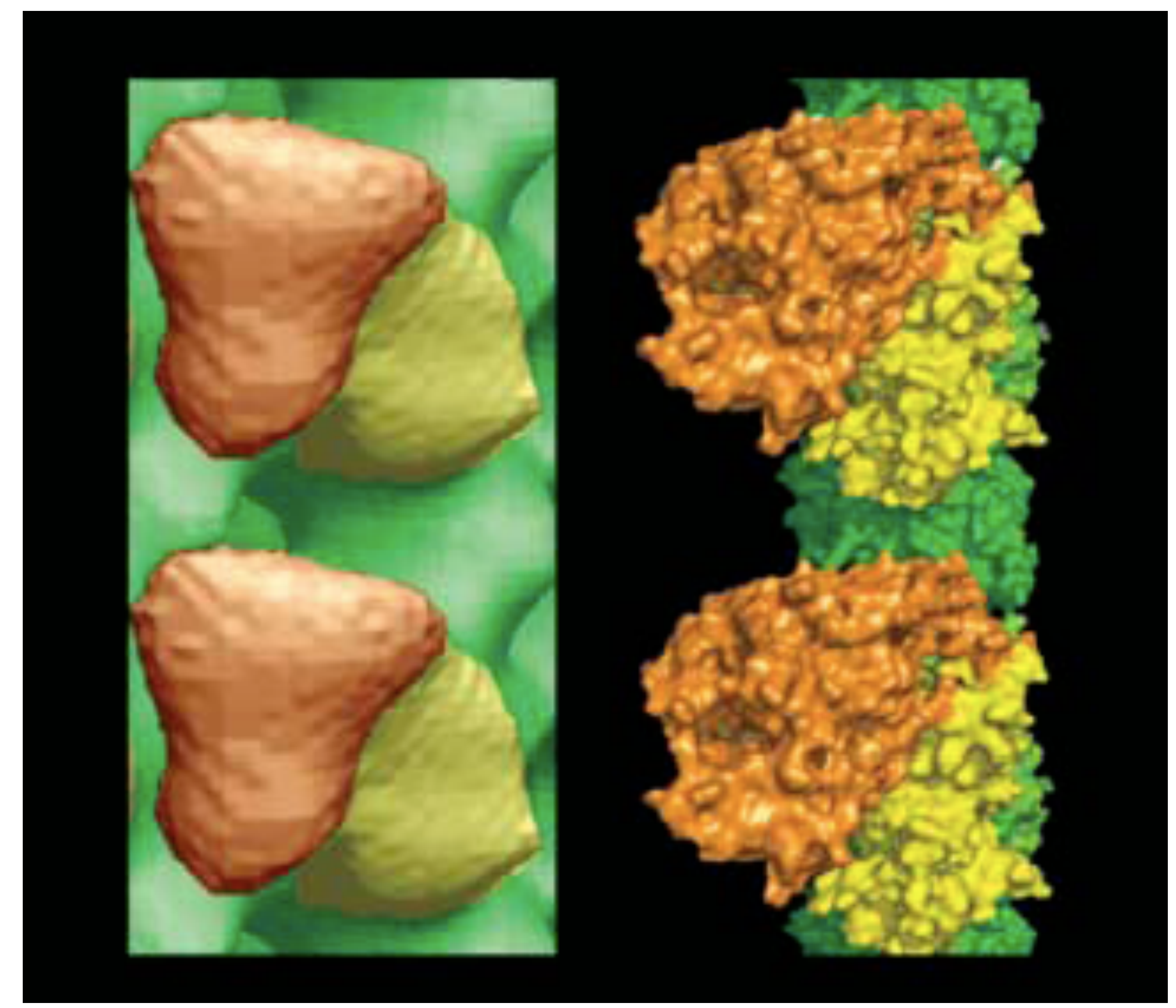
Q: When stepping processively, does kinesin spend most of its time in a two-heads bound (2HB) state or a one-head bound (1HB) state?

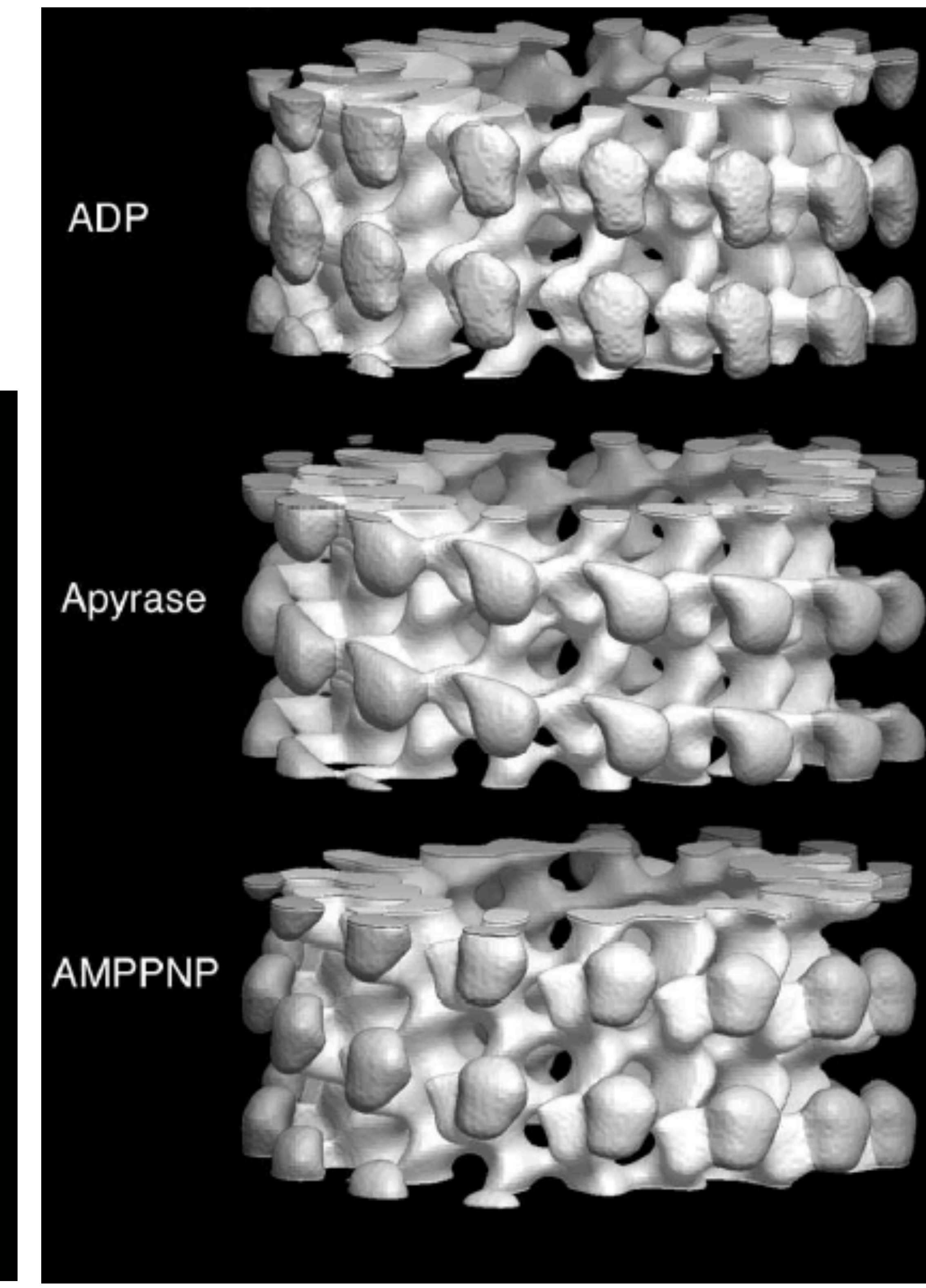
What is the nature of weakly-bound ADP state?



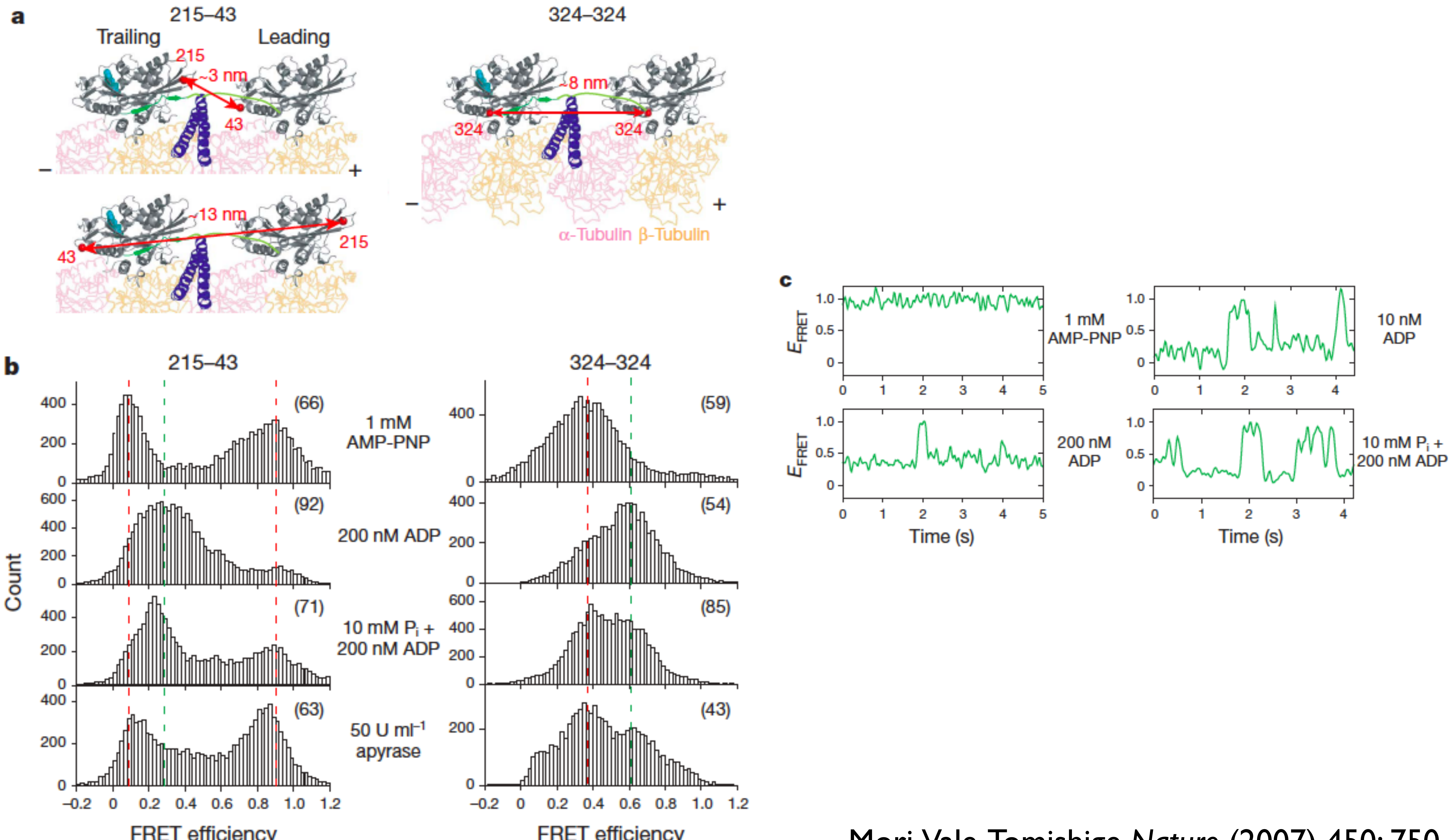




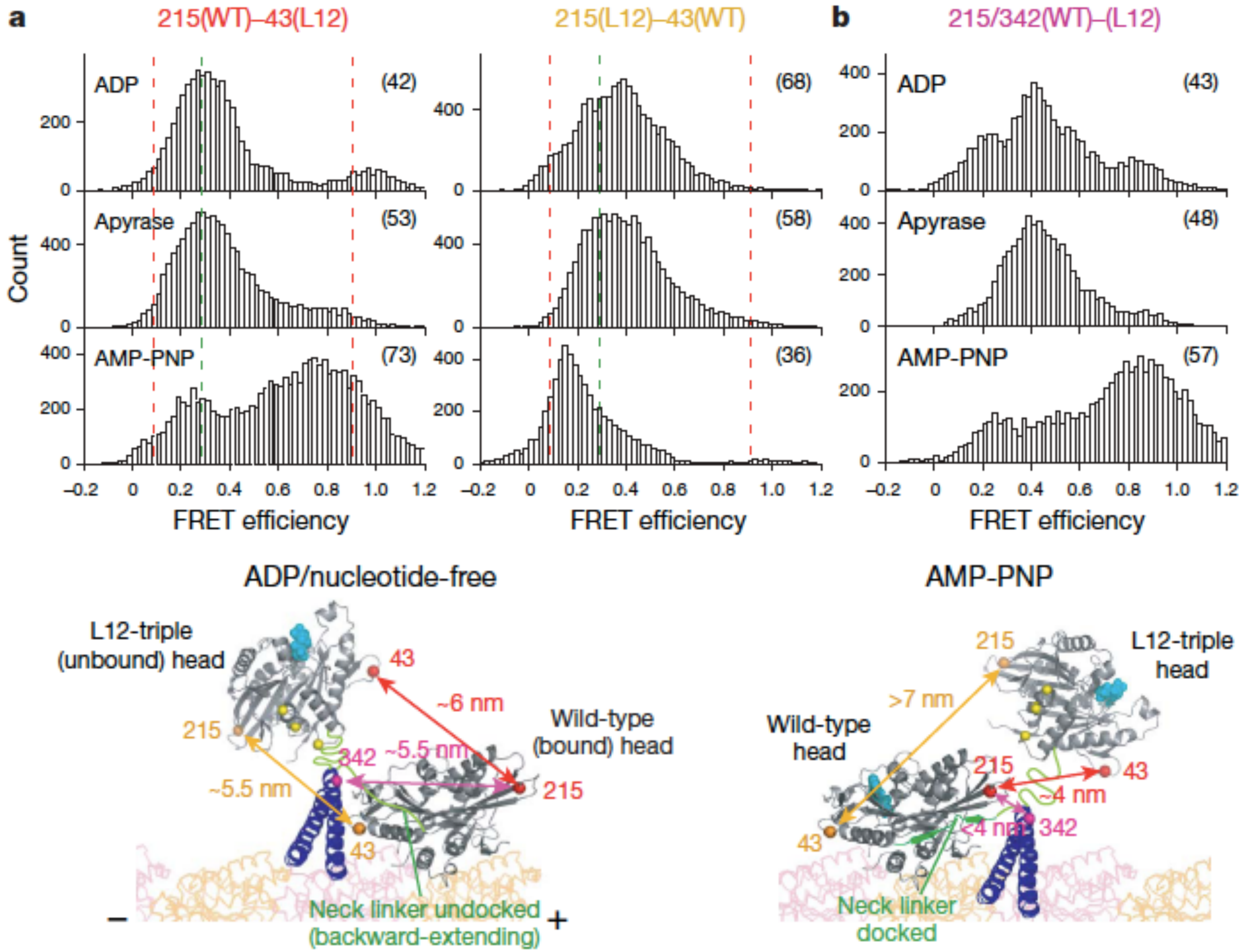


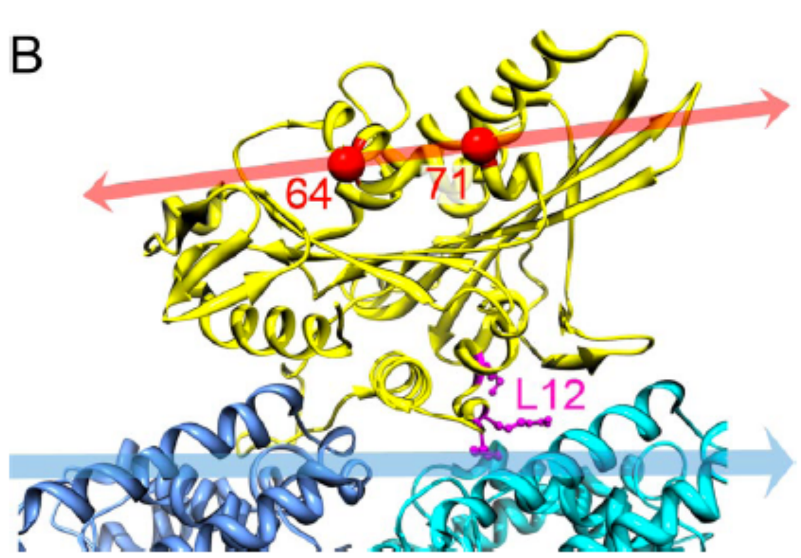


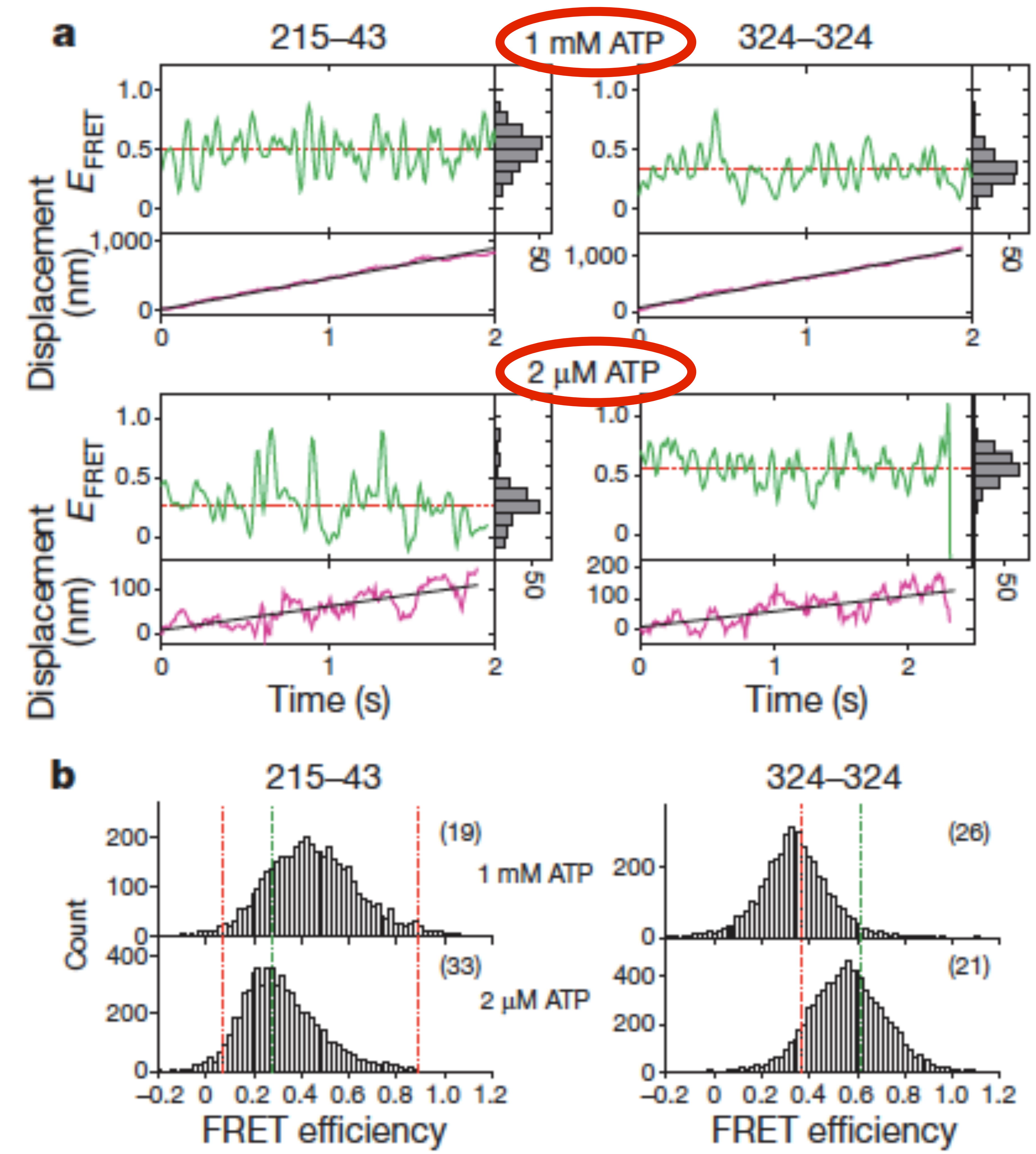
Cross and coworkers (2007) Science 316:120



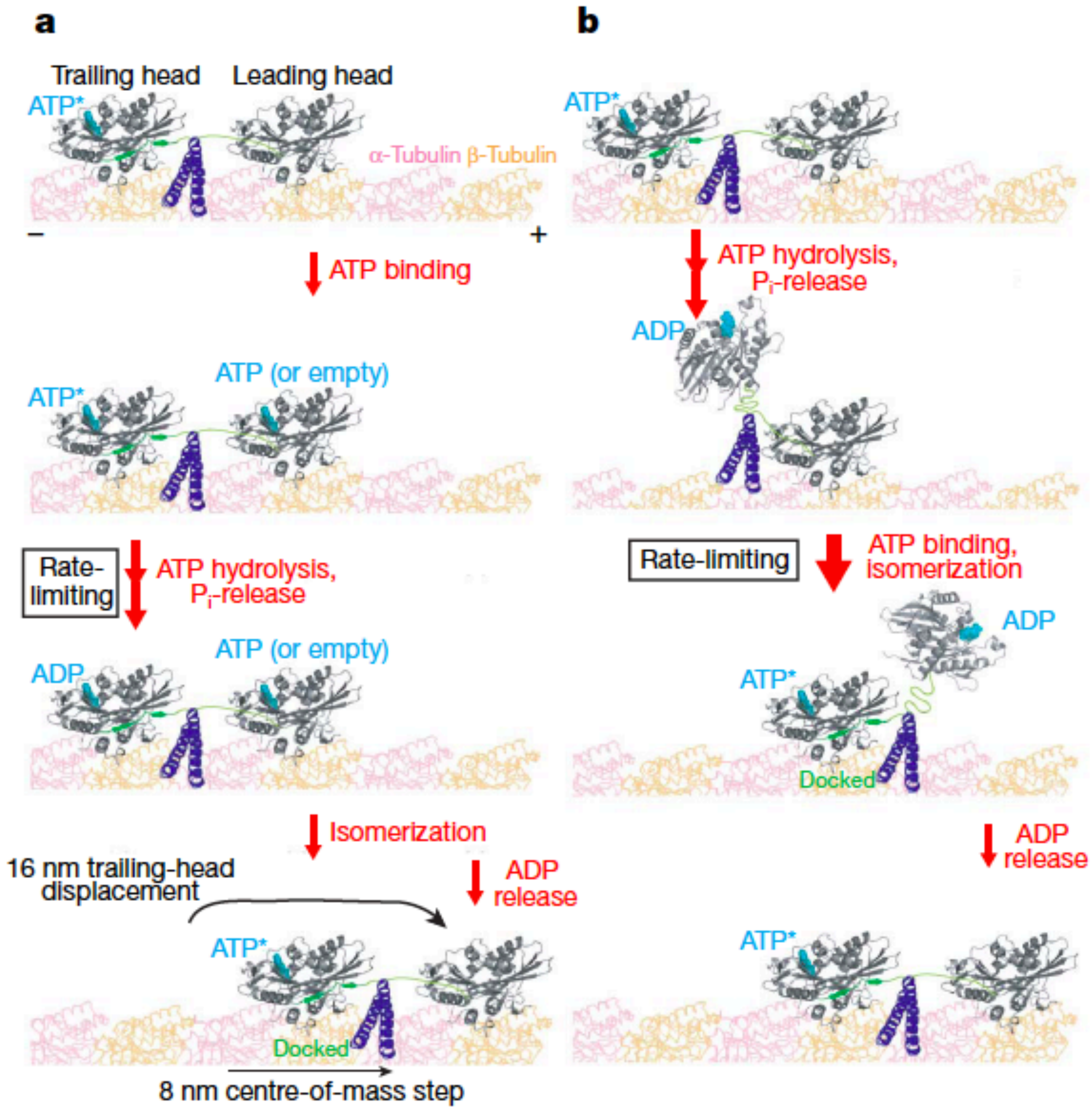
Mori, Vale, Tomishige *Nature* (2007) 450: 750



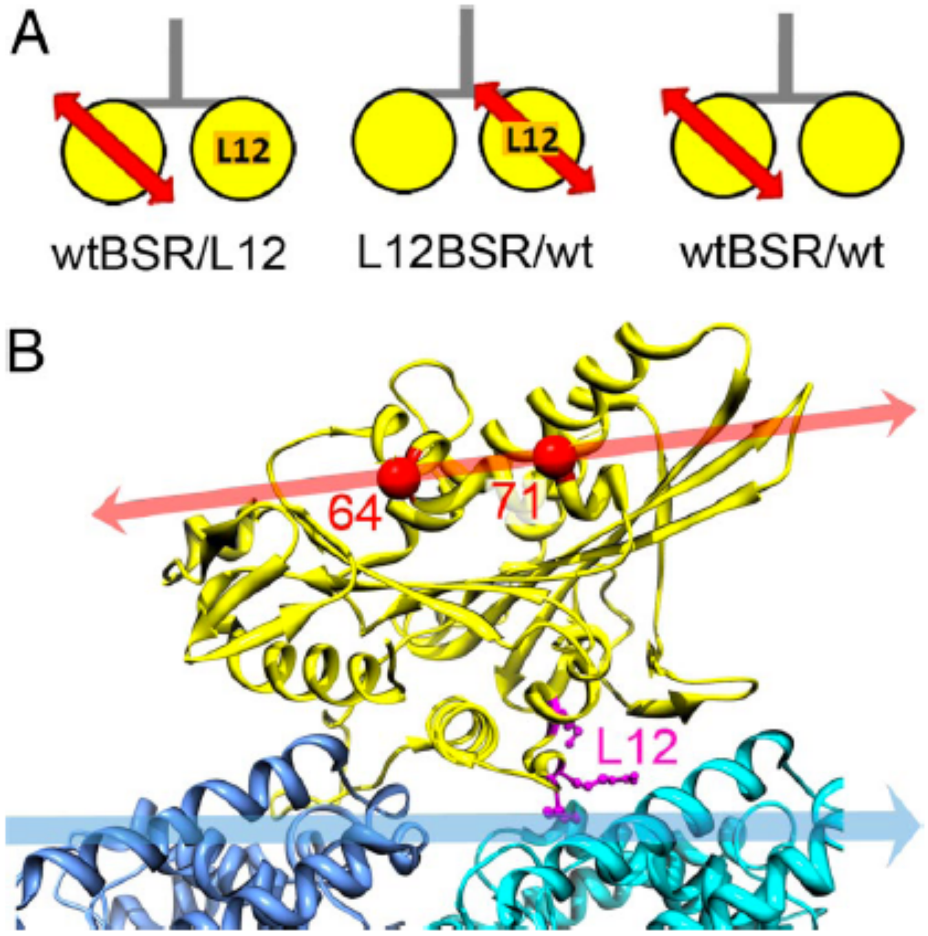




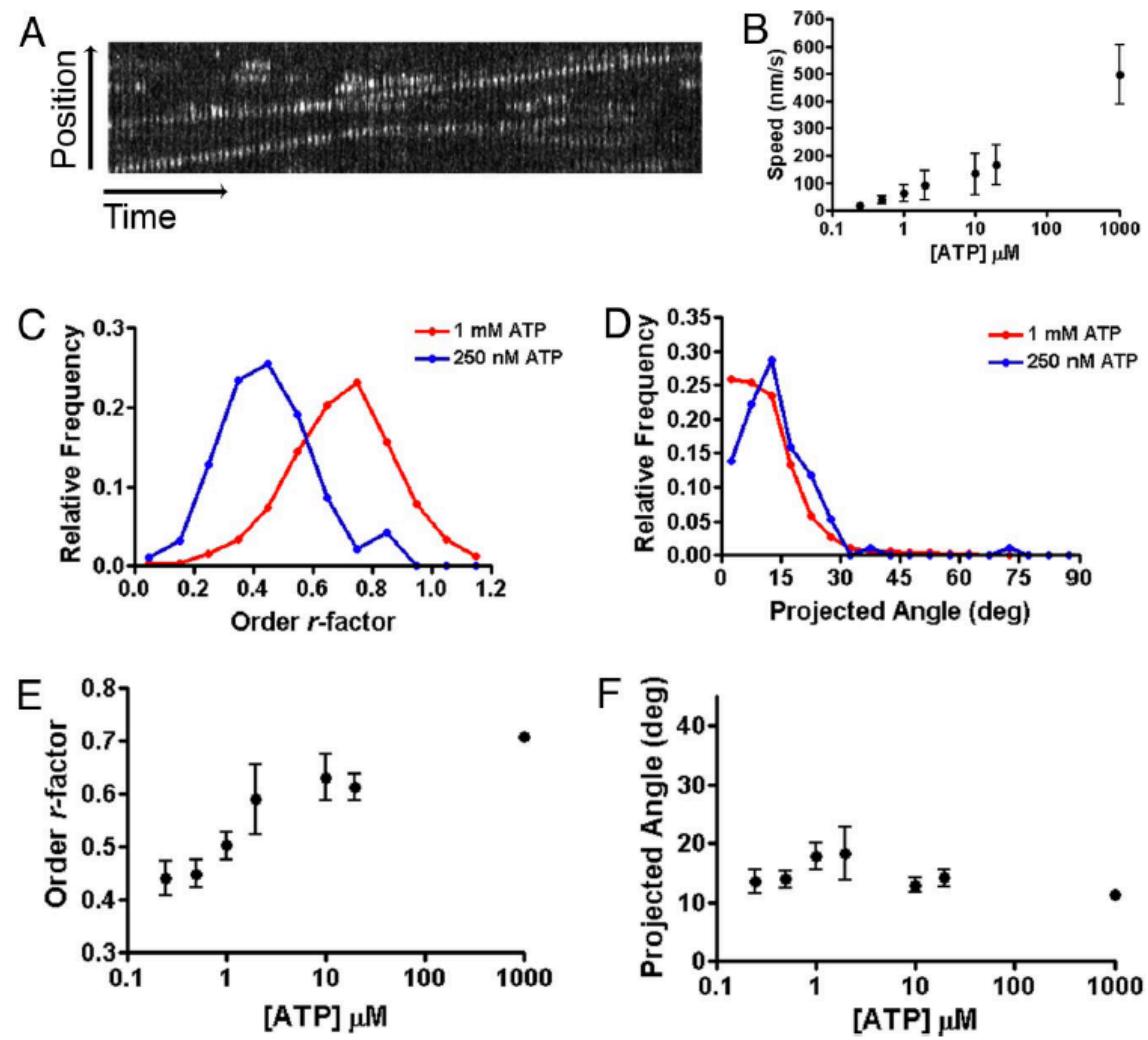
Mori, Vale, Tomishige *Nature* (2007) 450: 750



Mori, Vale, Tomishige *Nature* (2007) 450: 750

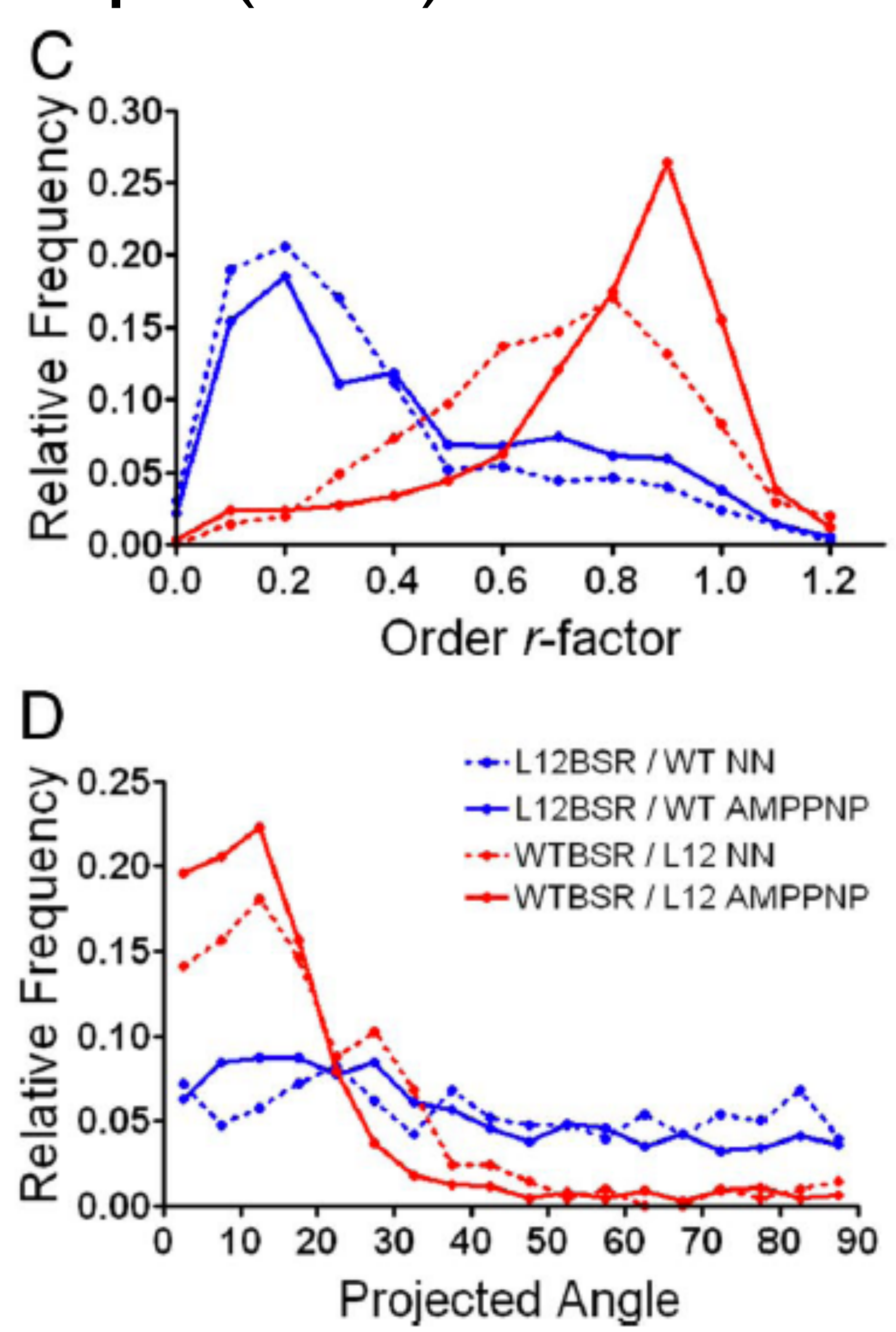


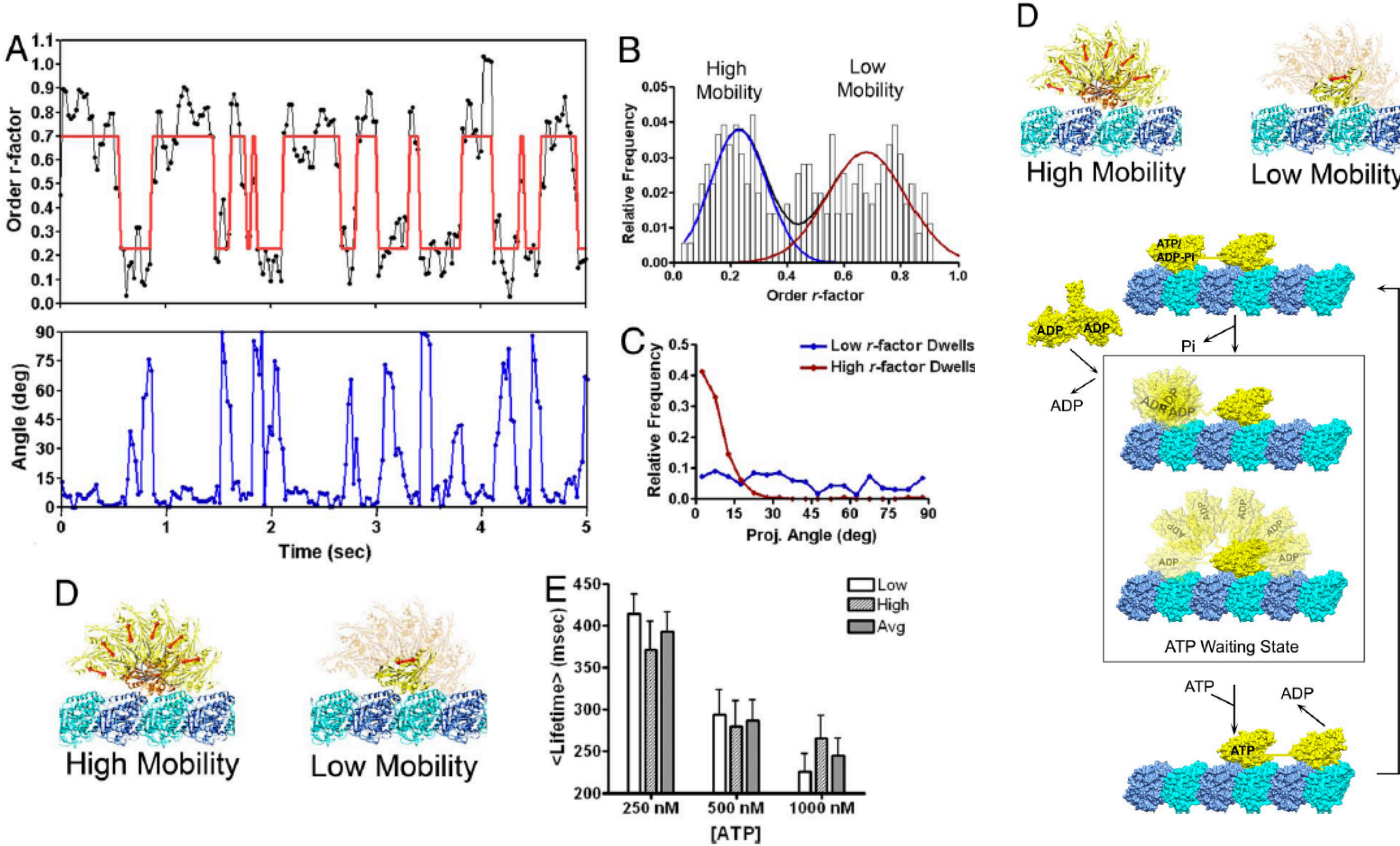
wt: wild type
BSR : label
L12 : mutant (MT binding reduced)



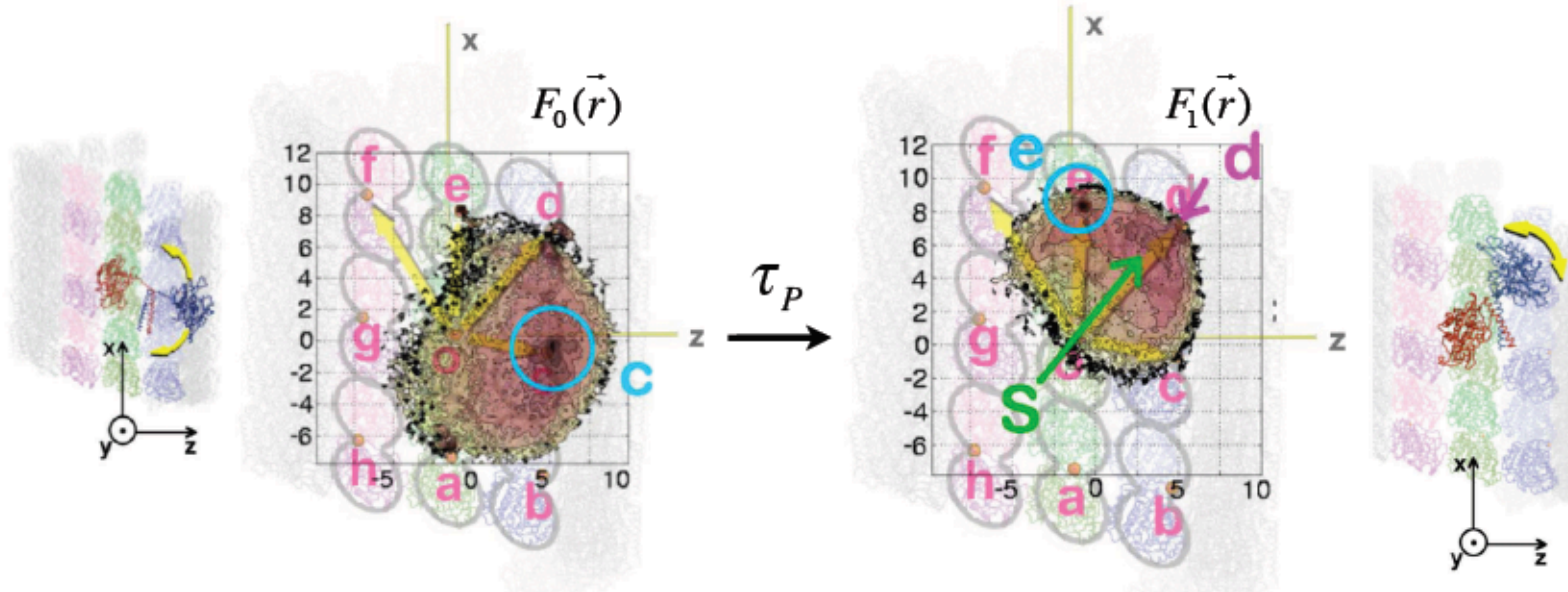
Fluorescence polarization microscope (FPM)

$-1<LD^0<1$

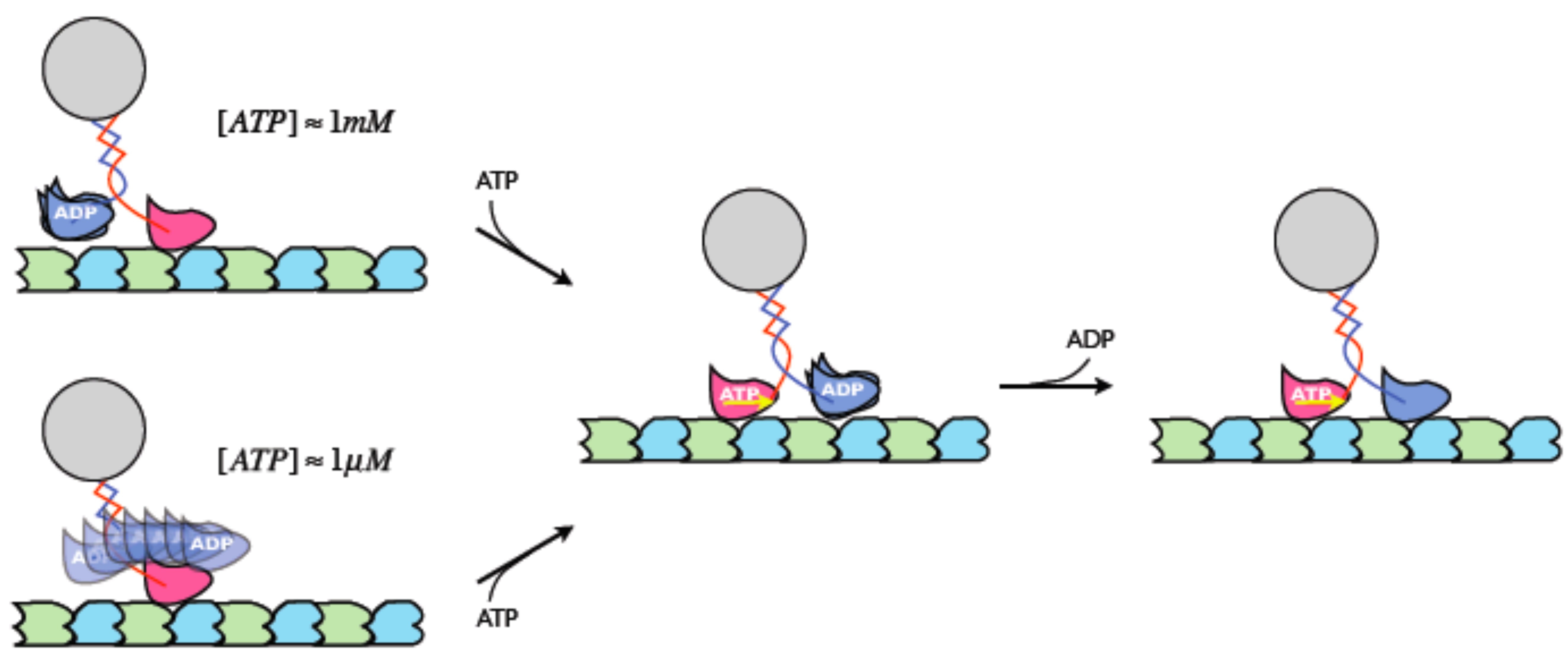




A
Order r-factor
Angle (deg)
Time (sec)
B
High Mobility
Low Mobility
Relative Frequency
Order r-factor
C
Low r-factor Dwells
High r-factor Dwells
Relative Frequency
Proj. Angle (deg)
D
High Mobility
Low Mobility
E
Low
High
Avg
<Lifetime> (msec)
250 nM
500 nM
1000 nM
[ATP]
D
High Mobility
Low Mobility
ATP/
ADP-Pi
ADP
ADP
Pi
ADP
ATP Waiting State
ATP
ADP
ATP

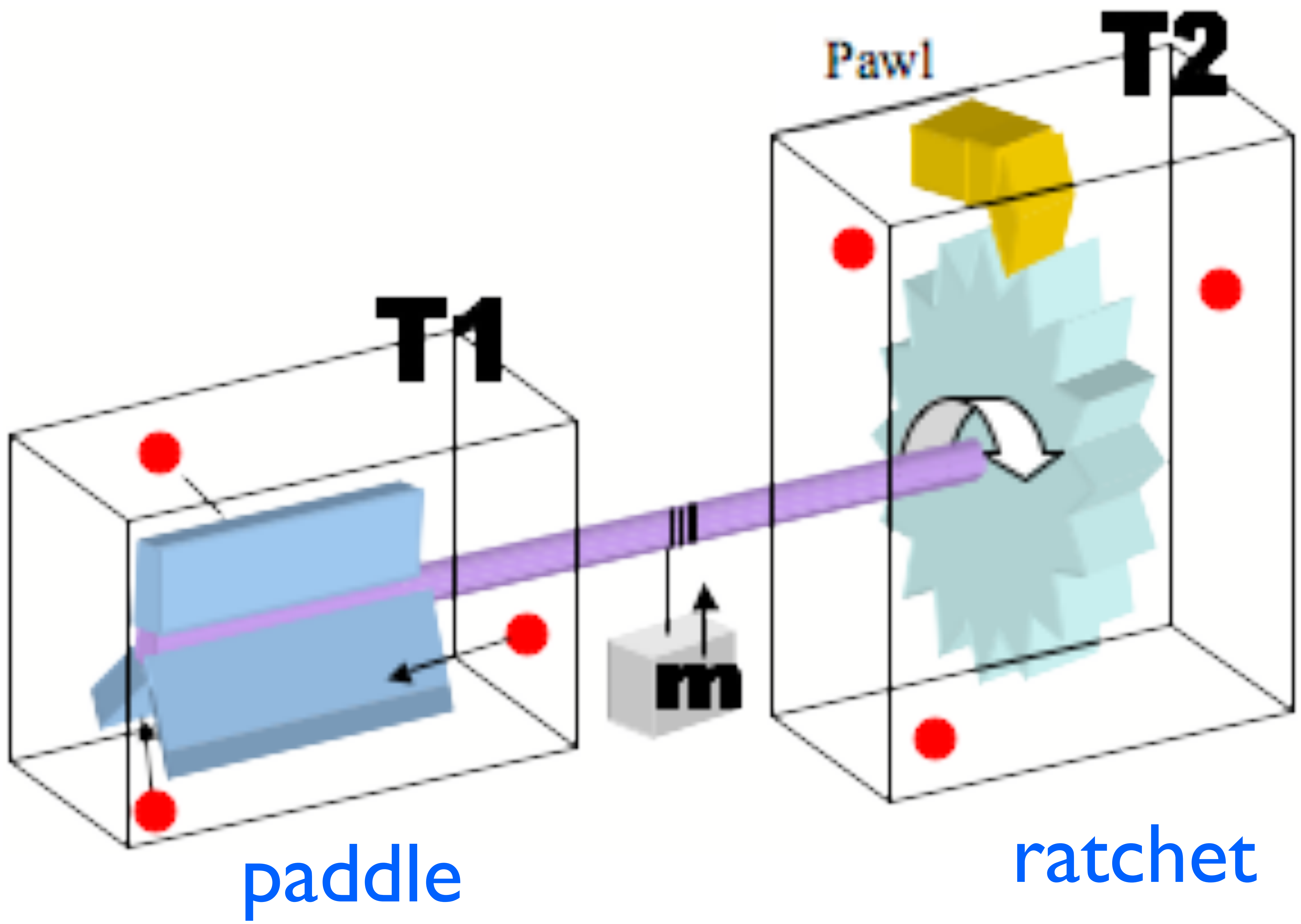


$F_0(\vec{r})$
τ_P
$F_1(\vec{r})$
x
z
y
f
e
d
g
c
h
a
b
S



$[ATP] \approx 1mM$
$[ATP] \approx 1\mu M$
ATP
ADP

Q: Does kinesin move by a power stroke or by a Brownian ratchet mechanism



Athermal fluctuation can induce a unidirectional transport in spatially asymmetric potential

Shaking-Induced Transition to a Nonequilibrium State

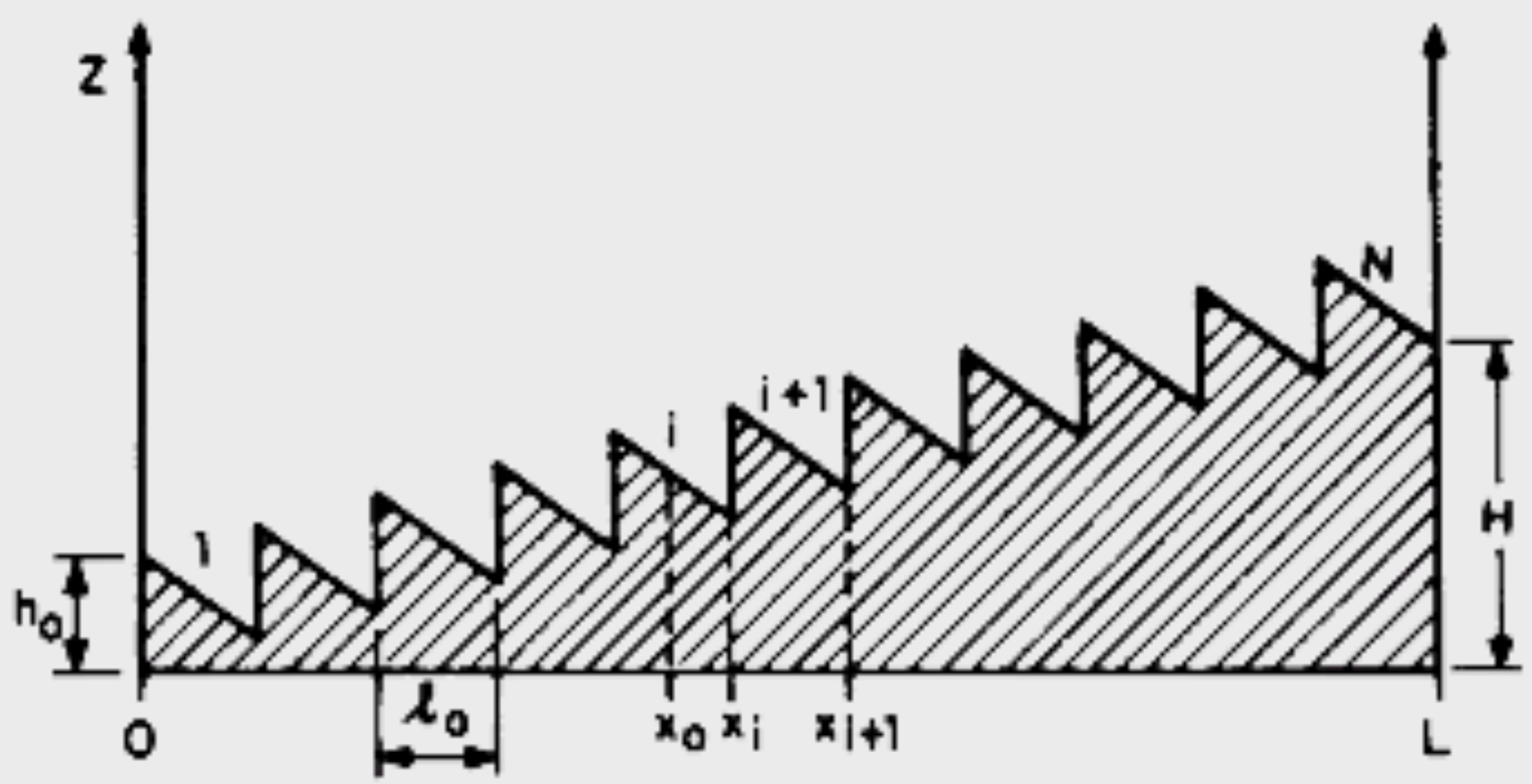


FIG. 1. Model potential is infinite at either end of, and below, ramp. Gravitational field and random force with friction ζ exist above ramp.

$$\rho(x,z,t) = (4\pi Dt)^{-1}\exp(-\{(x-x_0)^2 + [z - Z + (mg/\zeta)t]^2\}/4Dt),$$

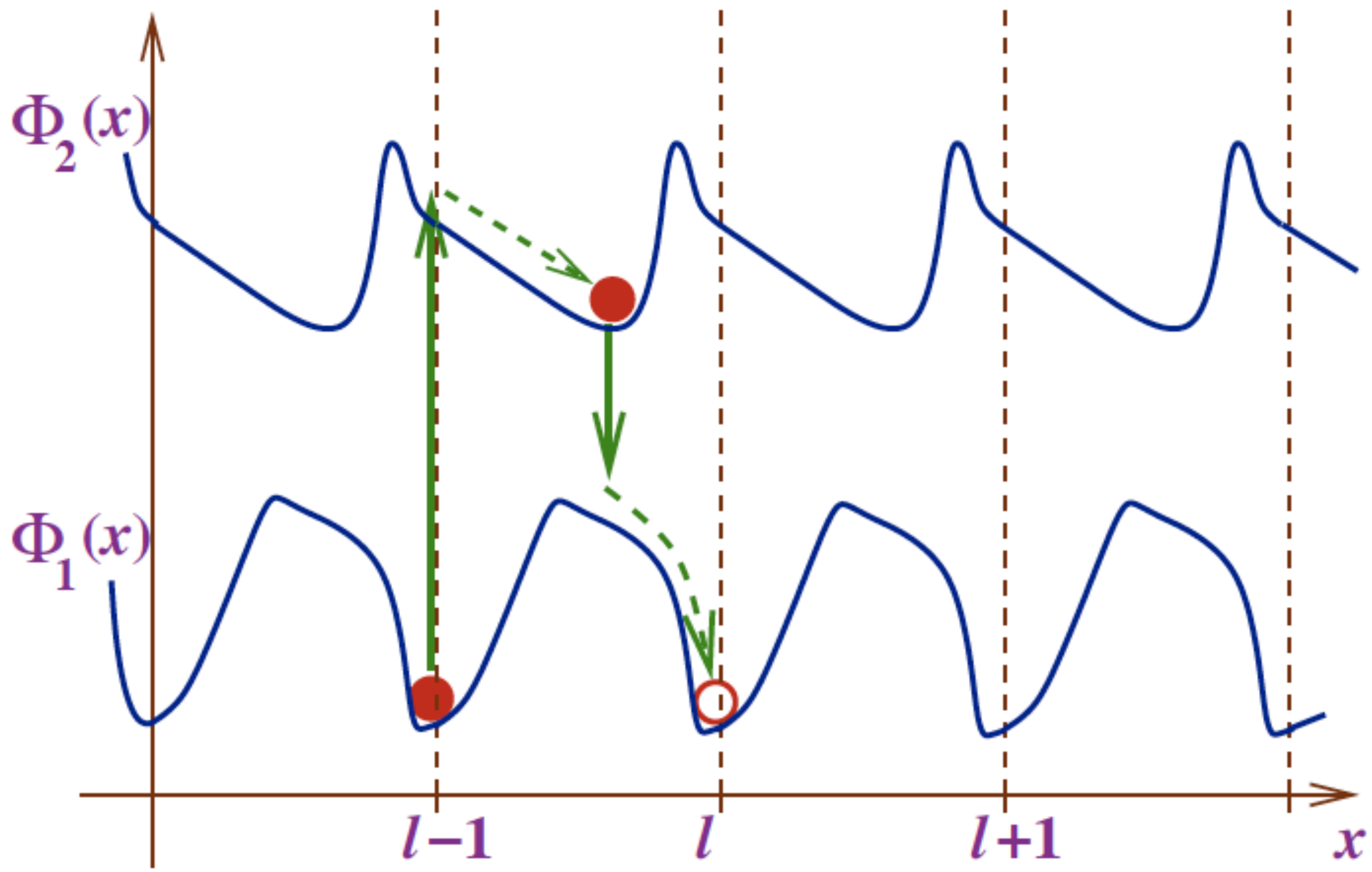
$$f_i = \left(\frac{mg\Delta z_i}{kT}\right)^{1/2} \frac{h_0}{l_0} \int_0^\infty \exp\left[-\left(\frac{mg\Delta z_i}{kT}\right)^{1/2} \frac{h_0}{l_0} y\right] \mathrm{erfc}(y)\,dy.$$

$dp_i/dt = k_{i-1}p_{i-1} - k_i p_i, \quad 1 < i < N;$

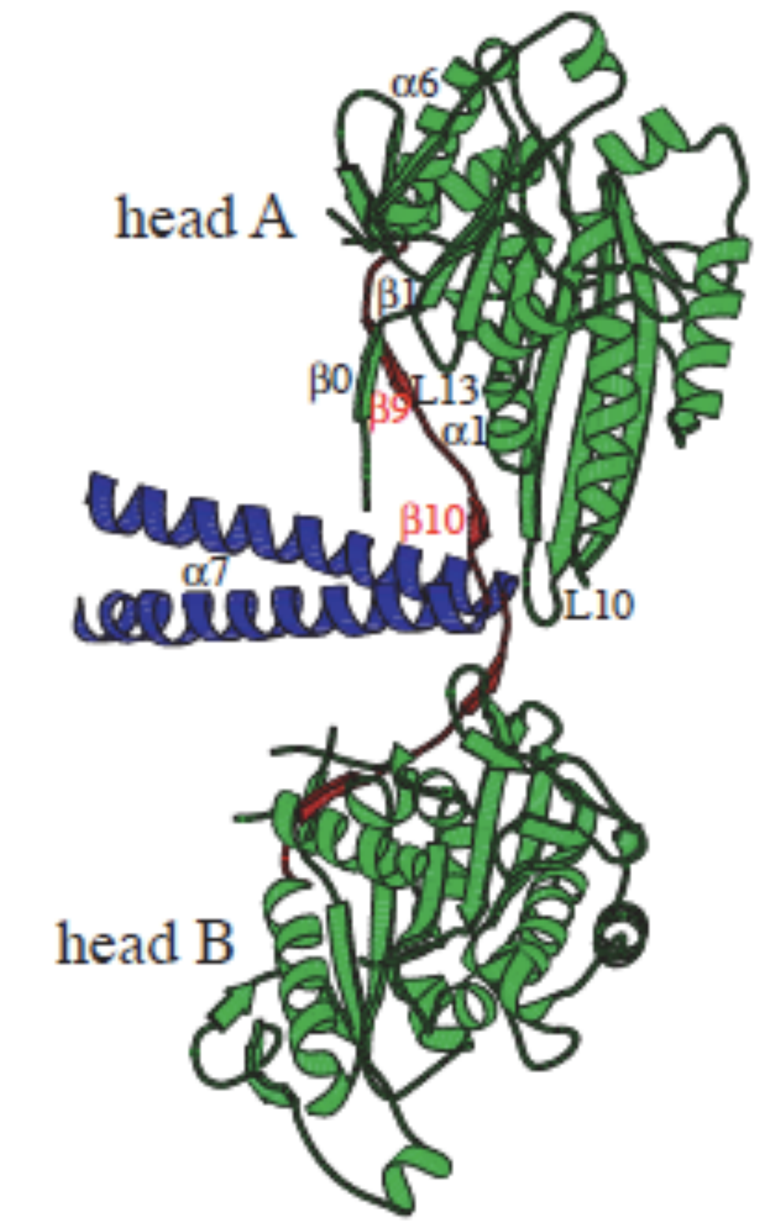
$dp_1/dt = -k_1 p_1; \quad dp_N/dt = k_{N-1}p_{N-1},$

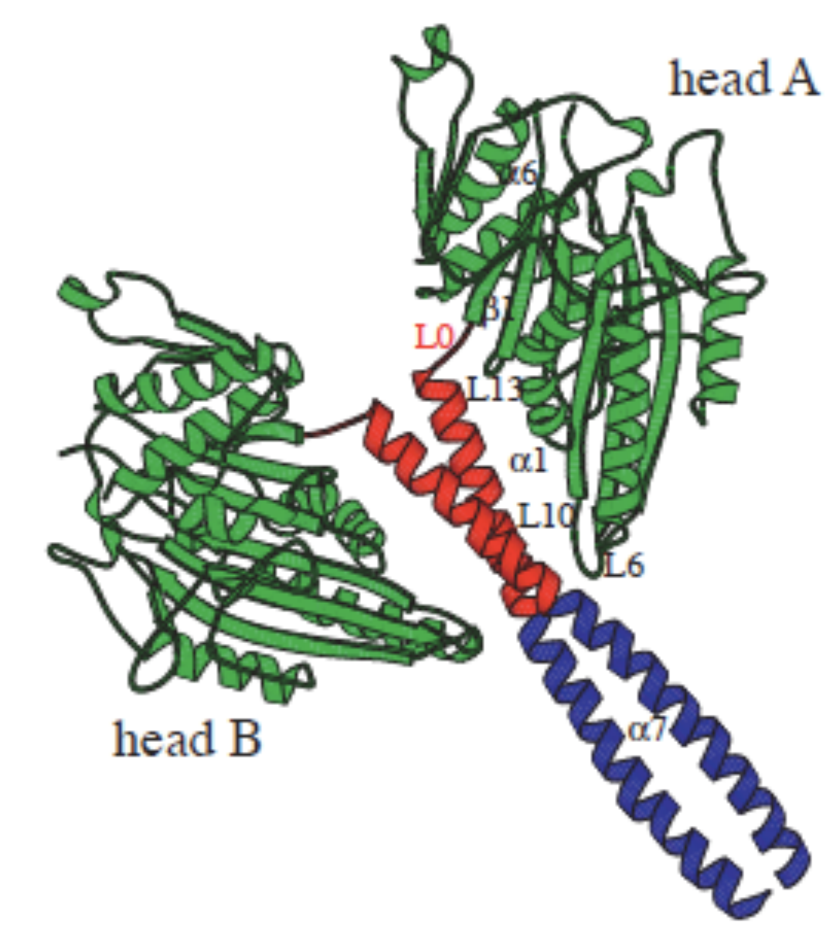
the uphill rate constant $k_i \equiv f_i/\tau$.

A.L.R. Bug and B.J. Berne (1987) PRL 59, 948

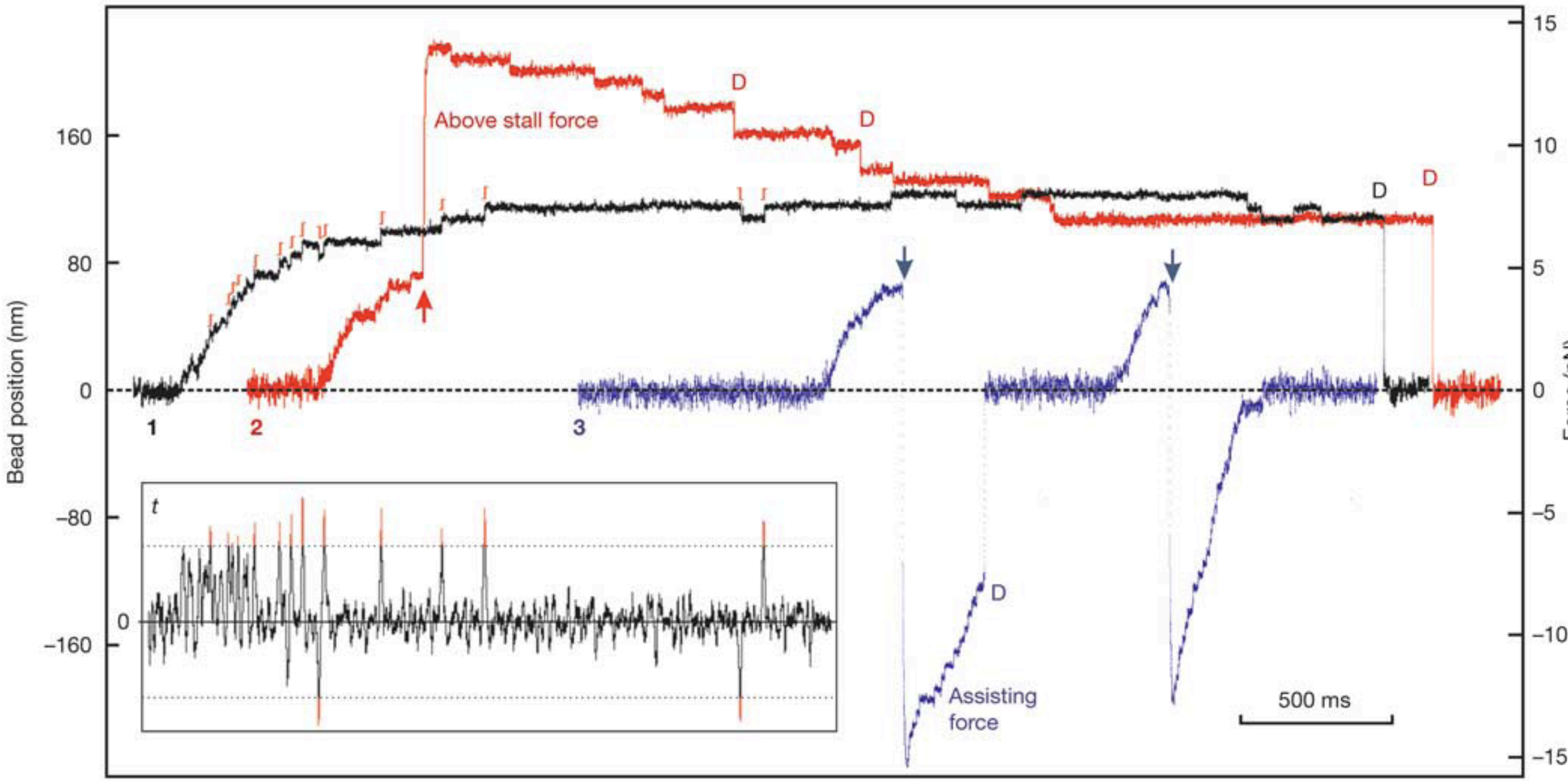


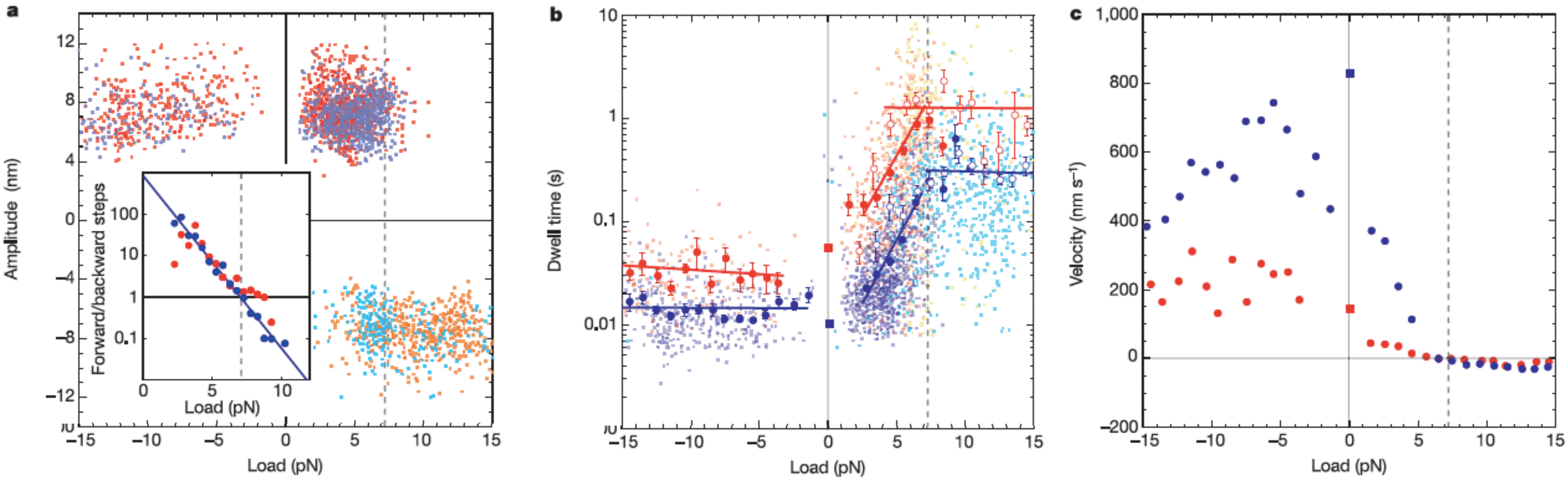
Kolomeisky & Fisher, Annu. Rev. Phys. Chem. (2007)





Q: Is the bacstepping cycle a reversal of the forward cycle, and does kinesin generate ATP under super-stall loads that force it to move backward?





forward load　　backward load

1mM ATP　　forward step
　　　　　　backward step

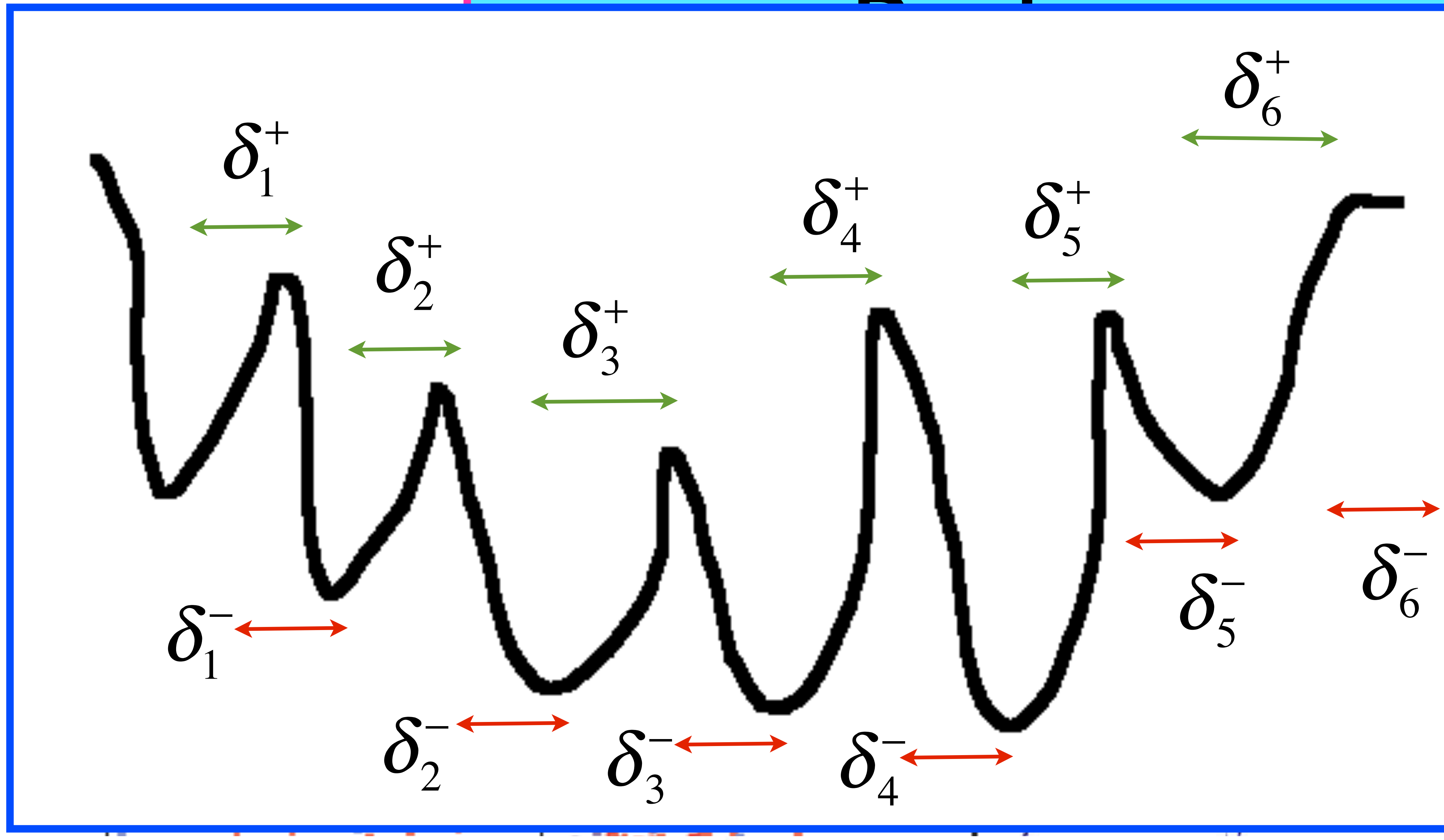
10μM ATP　　forward step
　　　　　　backward step

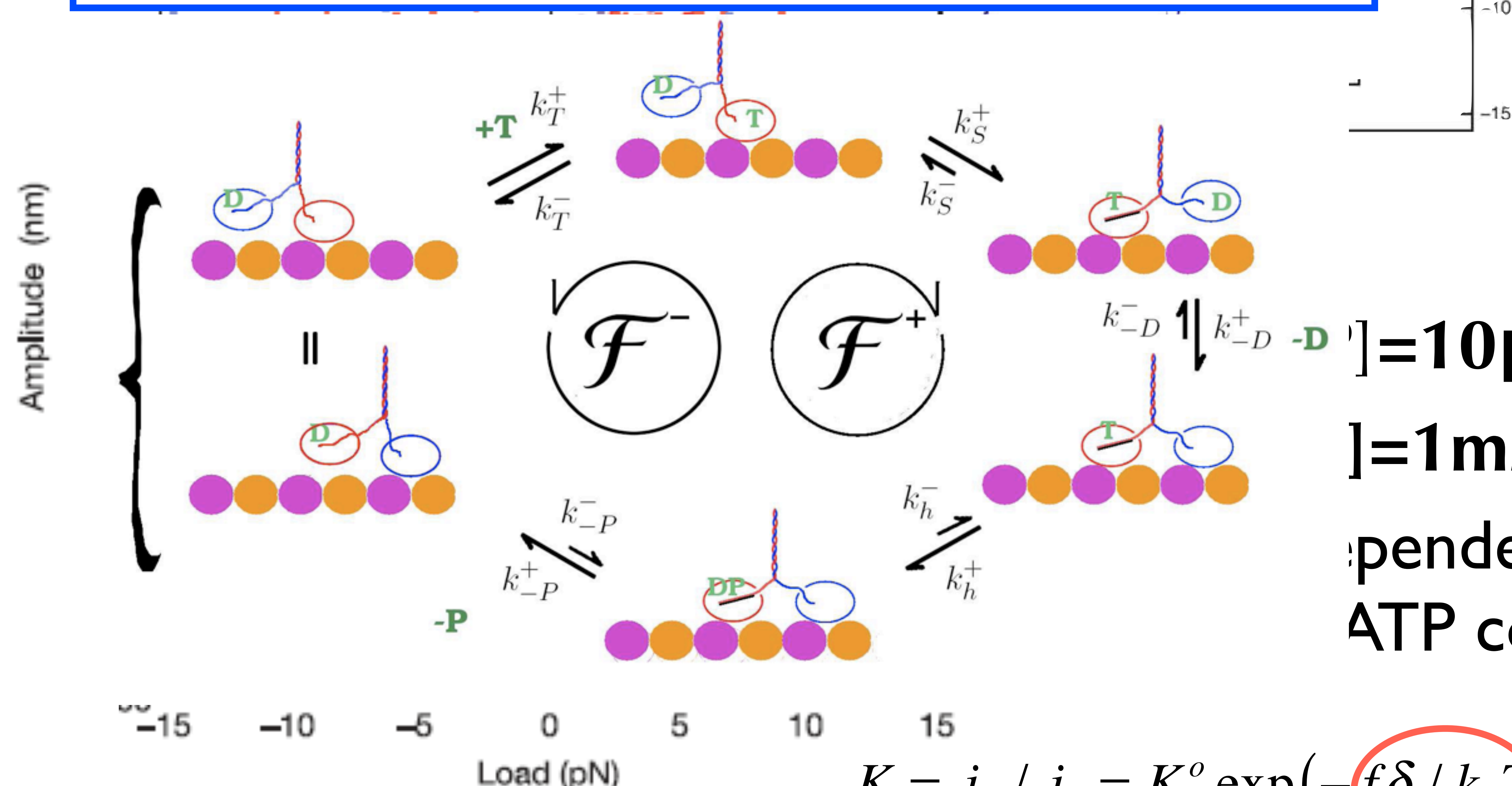
$$\frac{forward}{backward} = 802 \times \exp(-0.95 \times load)$$

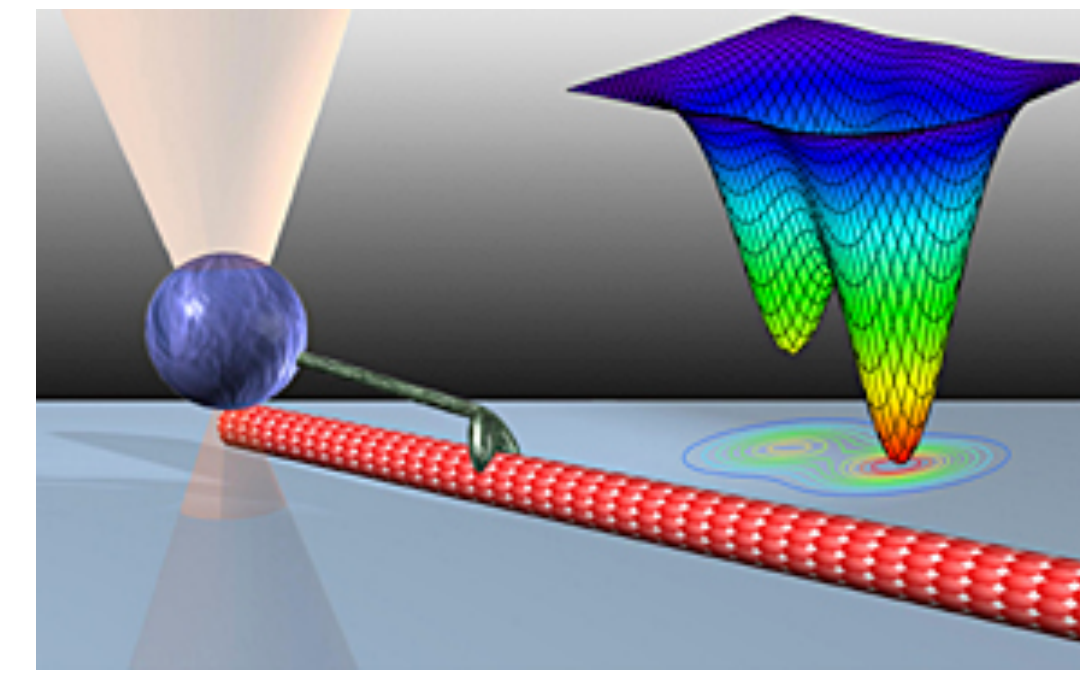
$$\frac{k_f}{k_b} = \frac{k_f^o e^{-f \times (x_0 - x_{TS})/k_B T}}{k_b^o e^{f \times (x_{TS} - x_{-1})/k_B T}} = \frac{k_f^o}{k_b^o} \exp\left[-f \times \frac{(x_0 - x_{-1})}{k_B T}\right]$$

der mechanical load

T experiments (Nature 2005)







$$\sum_i \delta_i^+ + \sum_i \delta_i^- = \delta = 8\ \text{nm}$$

$$\delta \approx 4\ \text{nm}$$

]=10μM

]=1mM

pendence of stall force

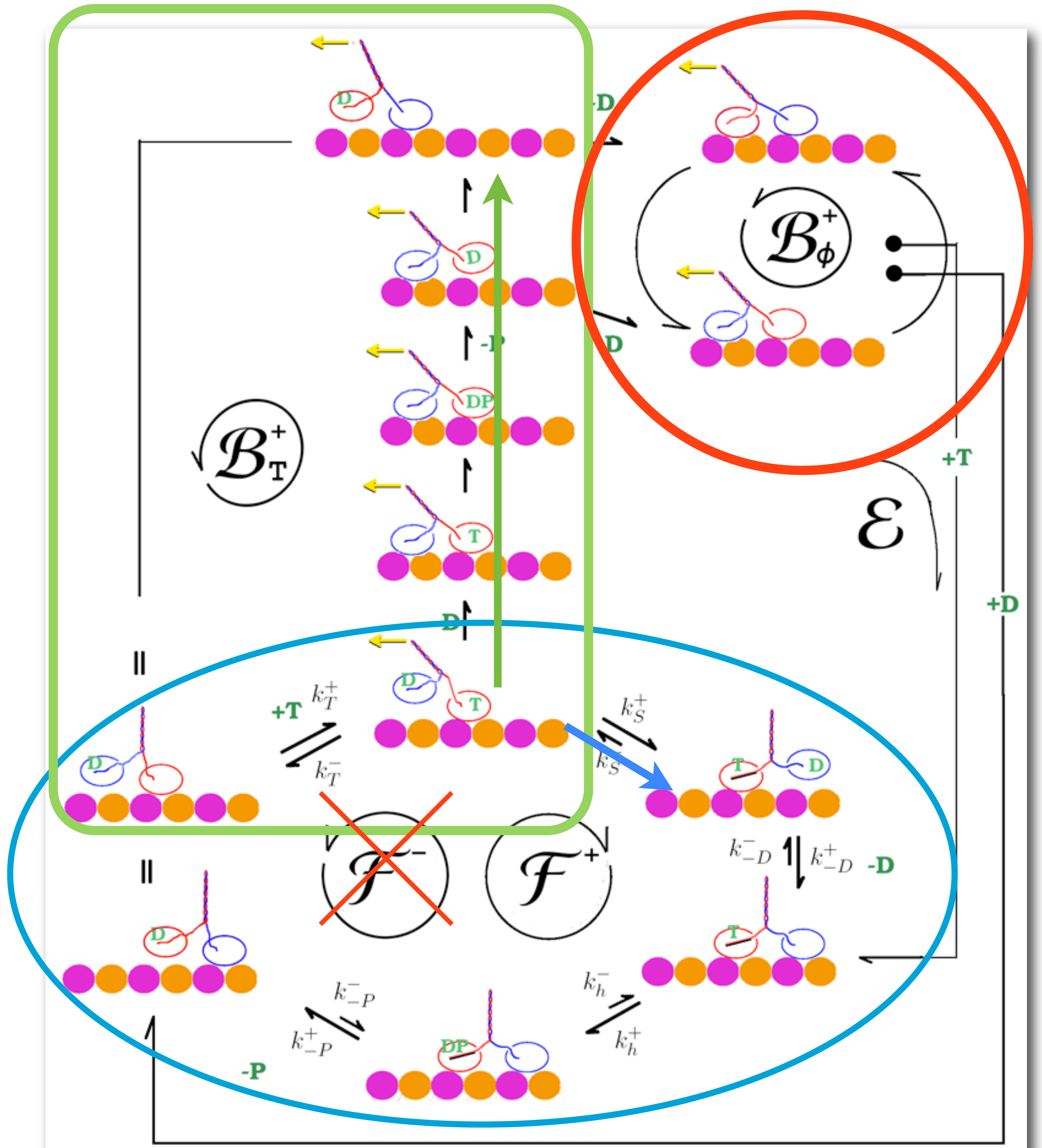
ATP concentration !!!

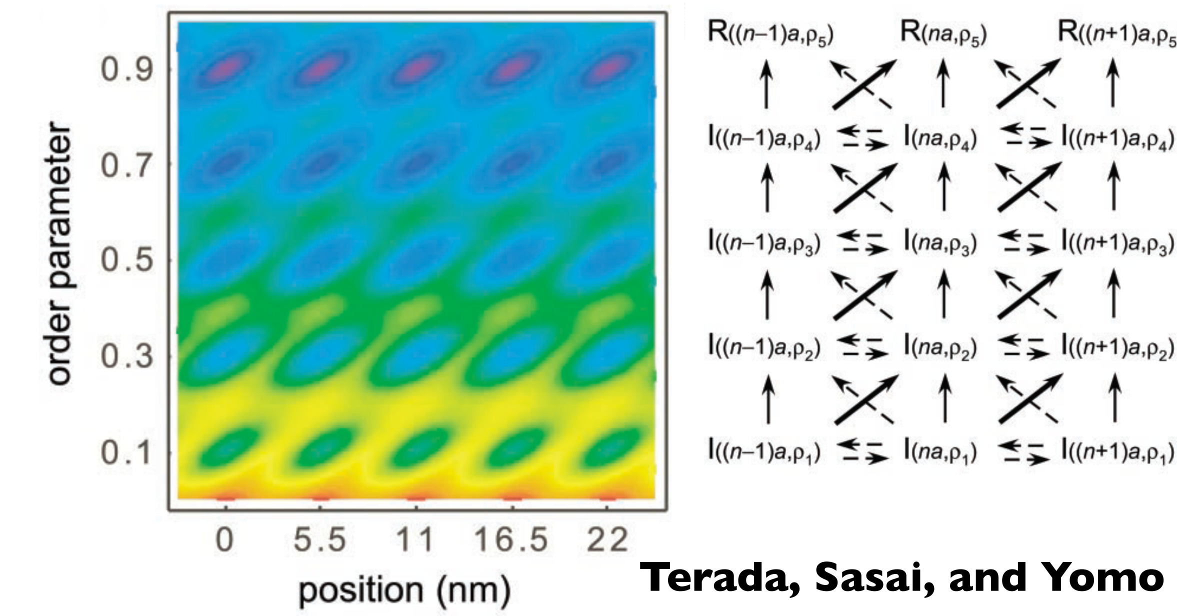
$$K = j_+ / j_- = K^o \exp(-f\delta / k_B T) = \left(K_{eq} \frac{[T]}{[D][P]} \right) \exp(-f\delta / k_B T)$$

K=1 (at stall condition)

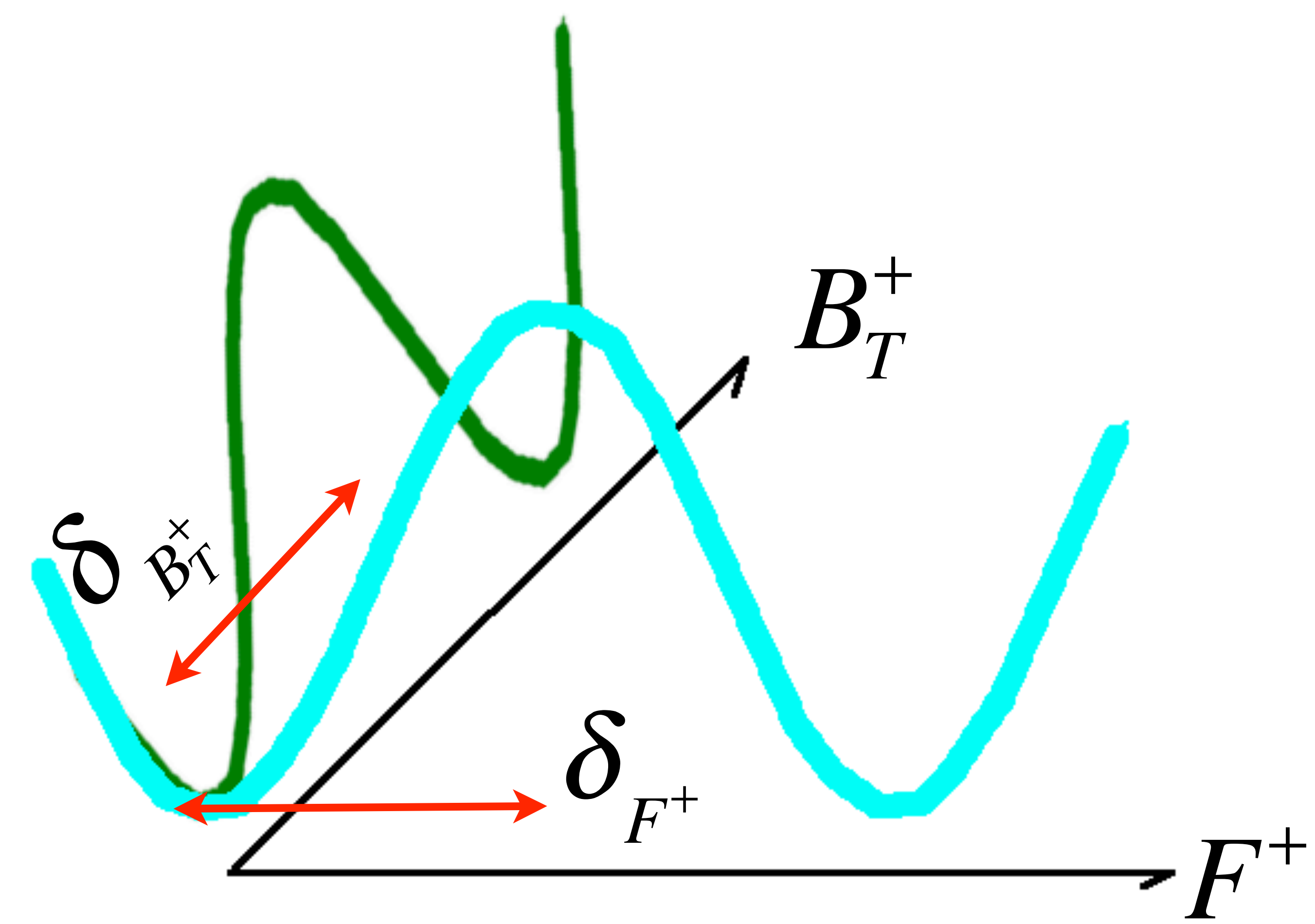
Multi-cycle model accounts for the backstep dynamics of kinesin motors

Hyeon, Klumpp & Onuchic (2009) *Phys. Chem. Chem. Phys.* 11:4899





Terada, Sasai, and Yomo (2006) PNAS



$$(\mathcal{F}^+ \oplus \mathcal{F}^- \oplus \mathcal{B}_T^+ \oplus \mathcal{B}_\phi^+)$$

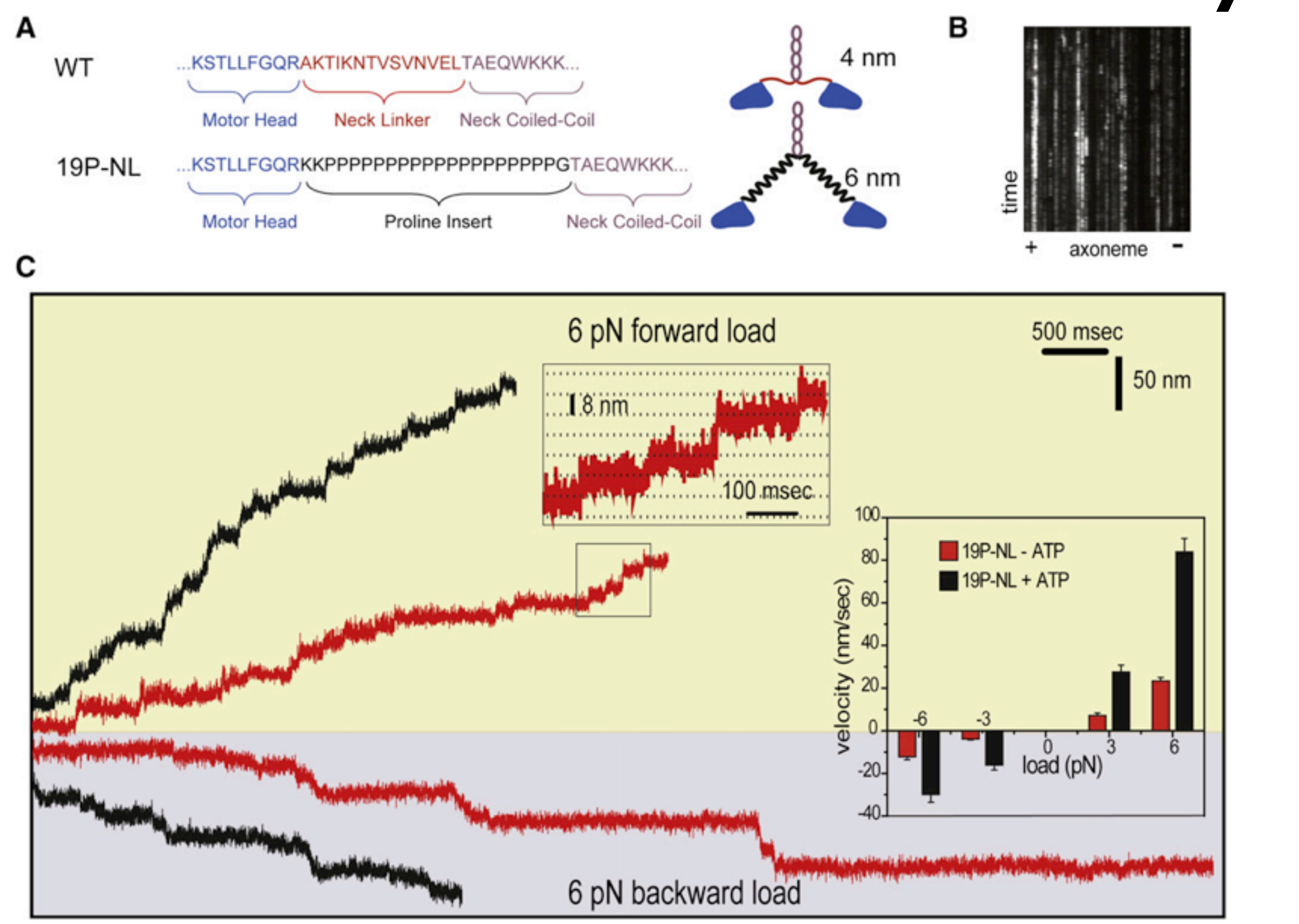
Vale and coworkers (2008) Cell

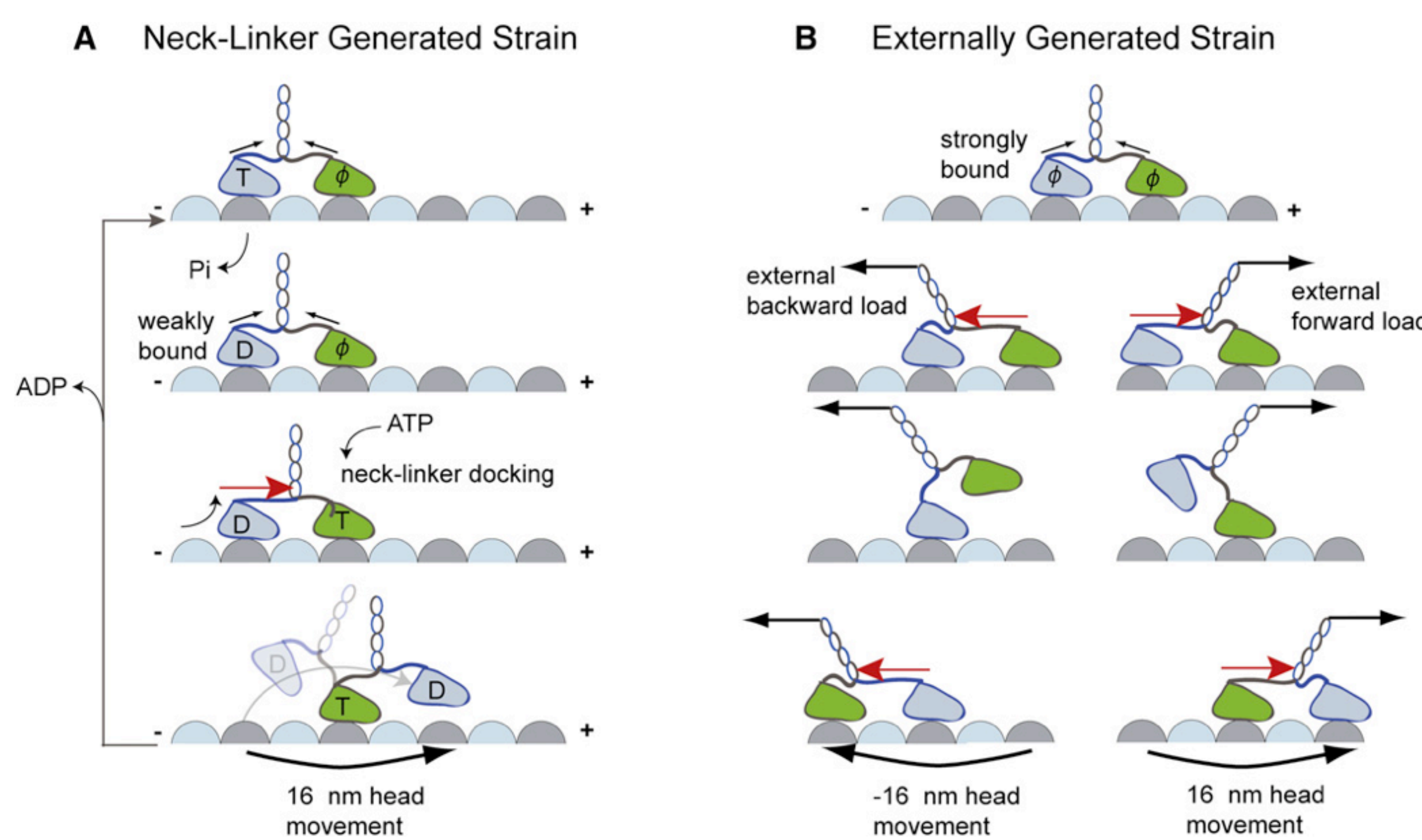
$$K_{\mathcal{F}^+\oplus\mathcal{B}_T^+}(f) = \frac{j_+}{j_-} = \frac{k_S^+}{k_{\mathcal{B}_T^+}^{eff}} \exp\left(-\frac{f(\delta_{\mathcal{F}^+} + \delta_{\mathcal{B}_T^+})}{k_B T}\right).$$

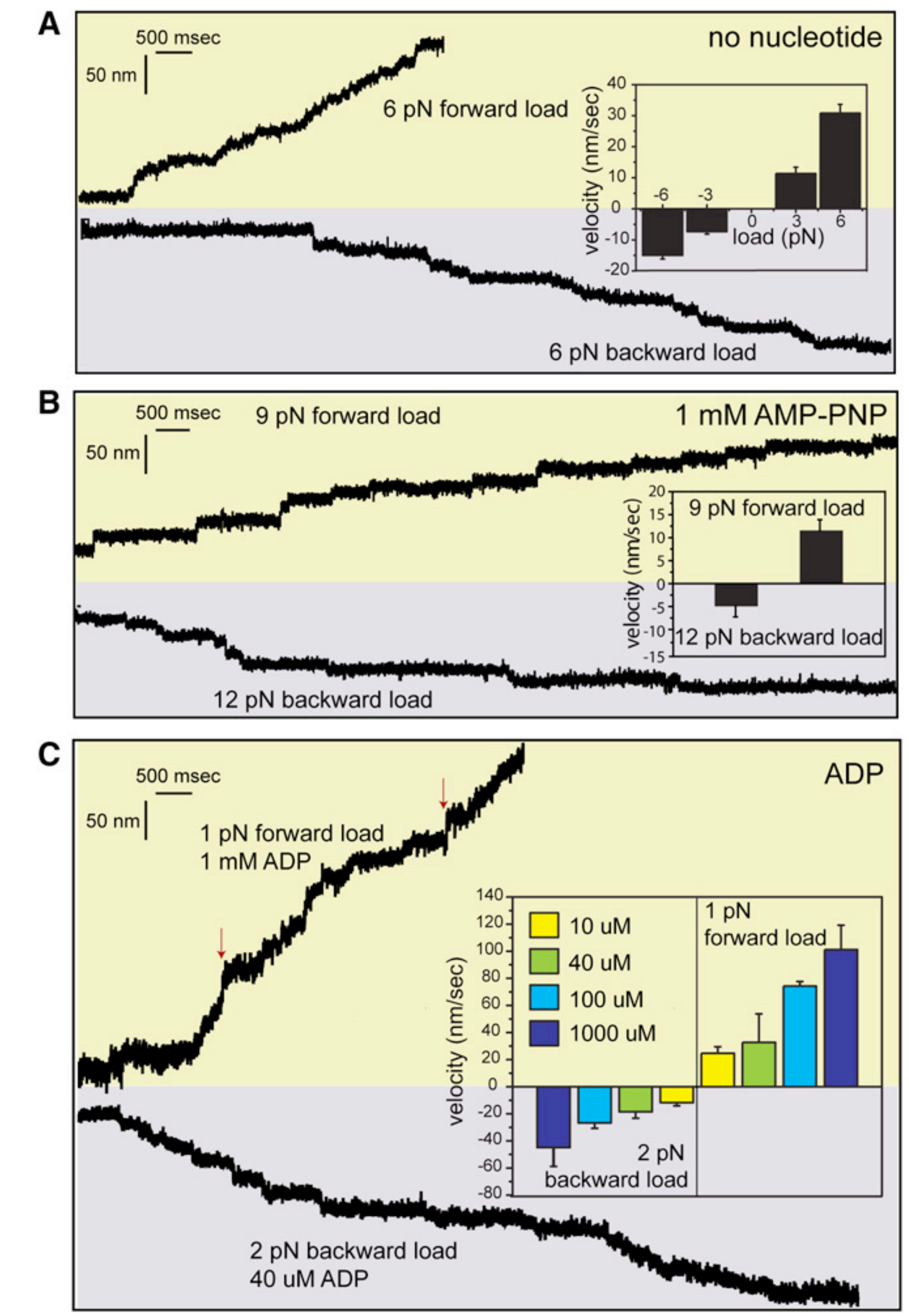
$$K_{eq}^o \equiv k_S^+ / k_{\mathcal{B}_T^+}^{eff}$$

$$f_{stall} = k_B T / (\delta_{\mathcal{F}^+} + \delta_{\mathcal{B}_T^+}) \times \log\left(k_S^+ / k_{\mathcal{B}_T^+}^{eff}\right)$$

Q: Conversely, when kinesin is sped up by an assisting force, is it going through its normal biochemical cycle or by some other pathway ?







Vale & coworkers,
Cell (2008) Vol 134, 1030-1041

KINETICS OF MOTOR PROTEINS

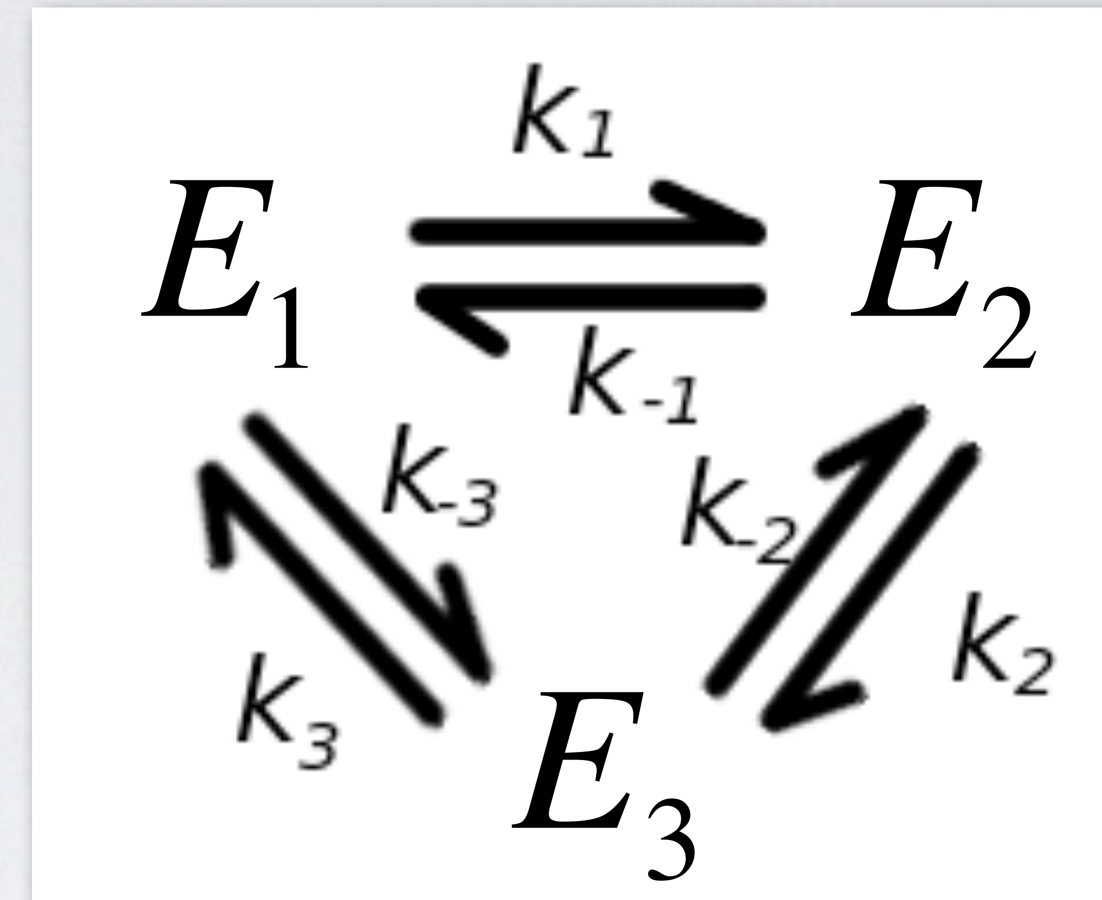
Steady State Solution for Reversible Cyclic Reaction

$$\frac{dP_{E_1}}{dt} = -(k_1^+ + k_3^-)P_{E_1} + k_1^- P_{E_2} + k_3^+ P_{E_3}$$

$$\frac{dP_{E_2}}{dt} = -(k_2^+ + k_1^-)P_{E_2} + k_2^- P_{E_3} + k_1^+ P_{E_1}$$

$$\frac{dP_{E_3}}{dt} = -(k_3^+ + k_2^-)P_{E_3} + k_3^- P_{E_1} + k_2^+ P_{E_2}$$

$$P_{E_1} + P_{E_2} + P_{E_3} = 1$$



$$V_3 \equiv k_i^+ P_{E_i}^{ss} - k_i^- P_{E_{i+1}}^{ss}$$

$$= \frac{k_1^+ k_2^+ k_3^+ - k_1^- k_2^- k_3^-}{k_1^+ k_2^+ + k_2^+ k_3^+ + k_3^+ k_1^+ + k_1^- k_2^- + k_2^- k_3^- + k_3^- k_1^- + k_1^- k_3^+ + k_1^+ k_2^- + k_2^+ k_3^-}$$

j_+ j_-

$$j(= V/\delta) = j_+ - j_-$$

$j = 0 \Rightarrow$ **Equilibrium**

$j \neq 0 \Rightarrow$ **Nonequilibrium Steady State**

$$k_1^+ \rightarrow k_T^+[T]$$

$$k_2^- \rightarrow k_{-P}^-[P]$$

$$k_3^- \rightarrow k_{-D}^-[D]$$

$$V = \delta \frac{\prod_{i=1}^{3} k_i^+[T] - \prod_{i=1}^{3} k_i^-[D][P]}{\sum\left(\left\{k_i^\pm\right\}\right)}$$

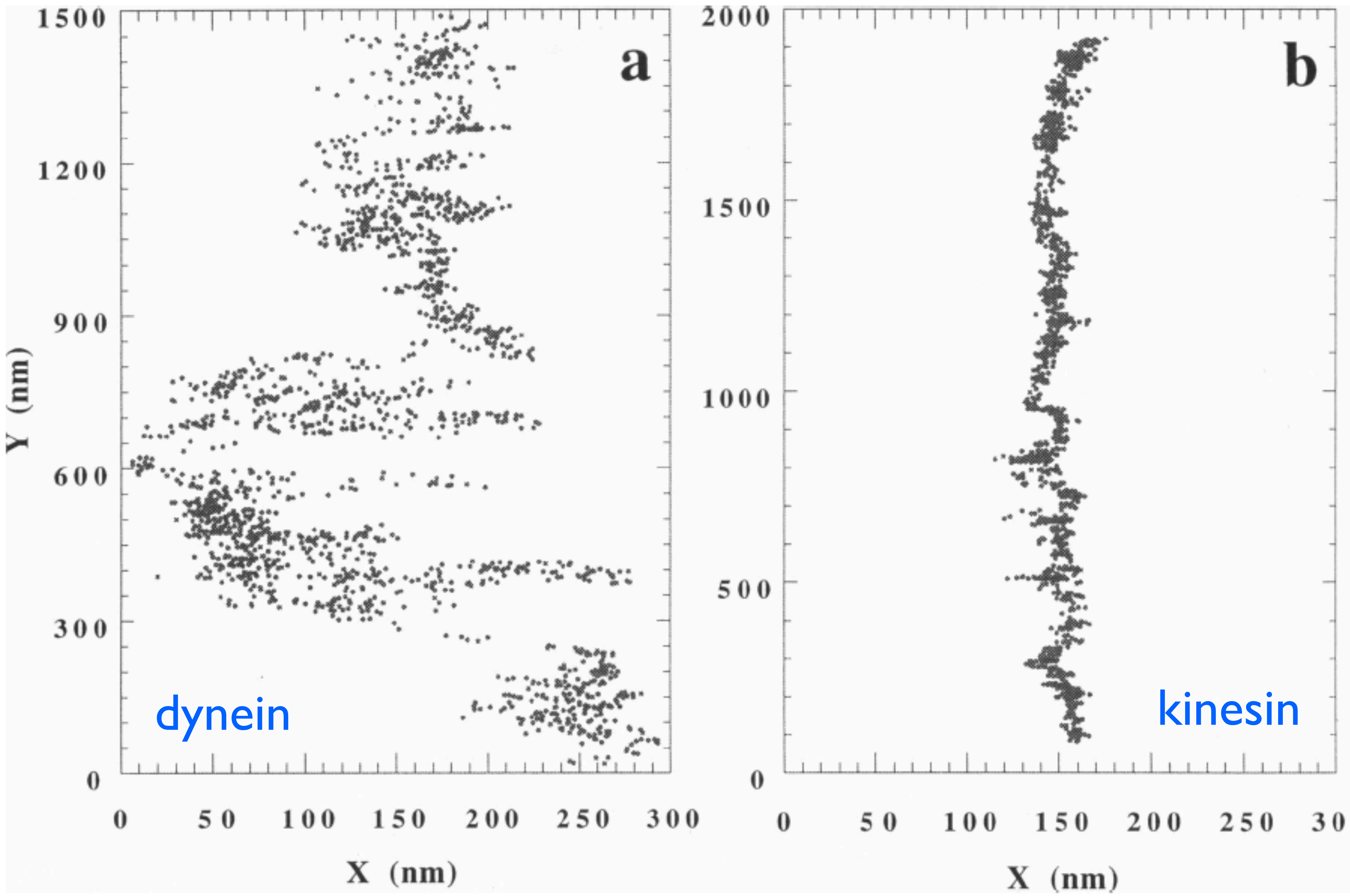
$$K = \frac{j_+}{j_-} = \frac{k_1^+ k_2^+ k_3^+ [T]}{k_1^- k_2^- k_3^- [D][P]}$$

in the presence of backward load

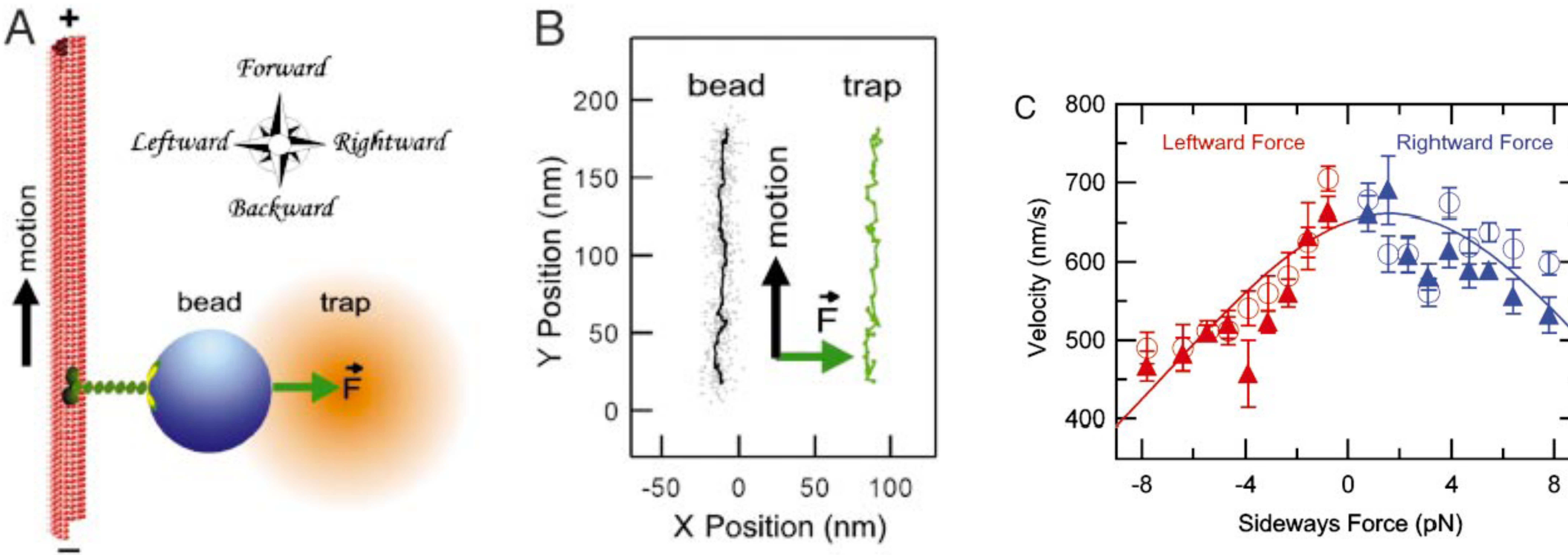
$$k_i^+ \rightarrow k_i^+ \exp\left(-f\delta_i / k_B T\right)$$

$$k_i^+ \rightarrow k_i^- \exp\left(+f\delta_i / k_B T\right)$$

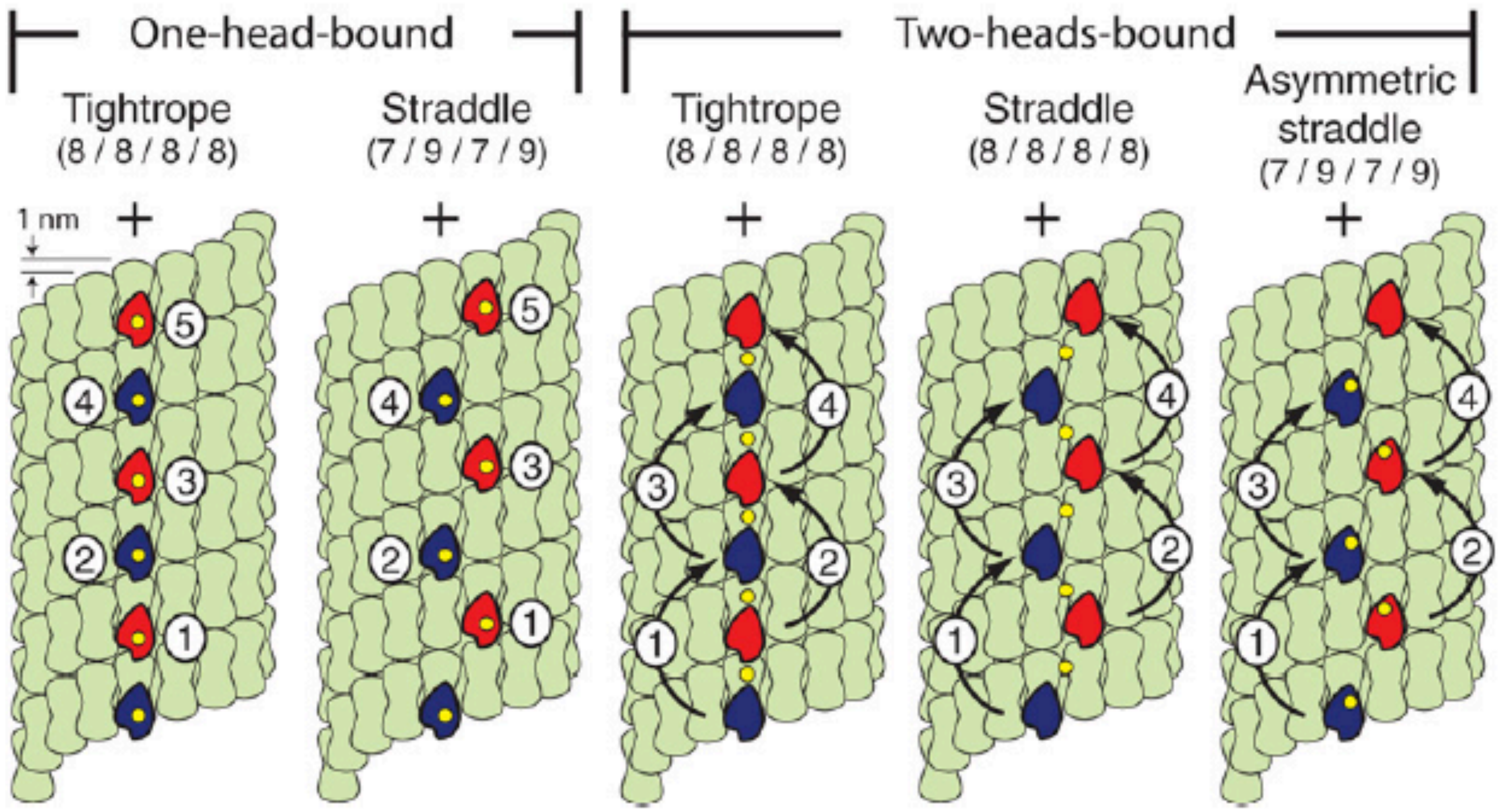
Q: How does kinesin manage to track parallel to a single protofilament of the MT?

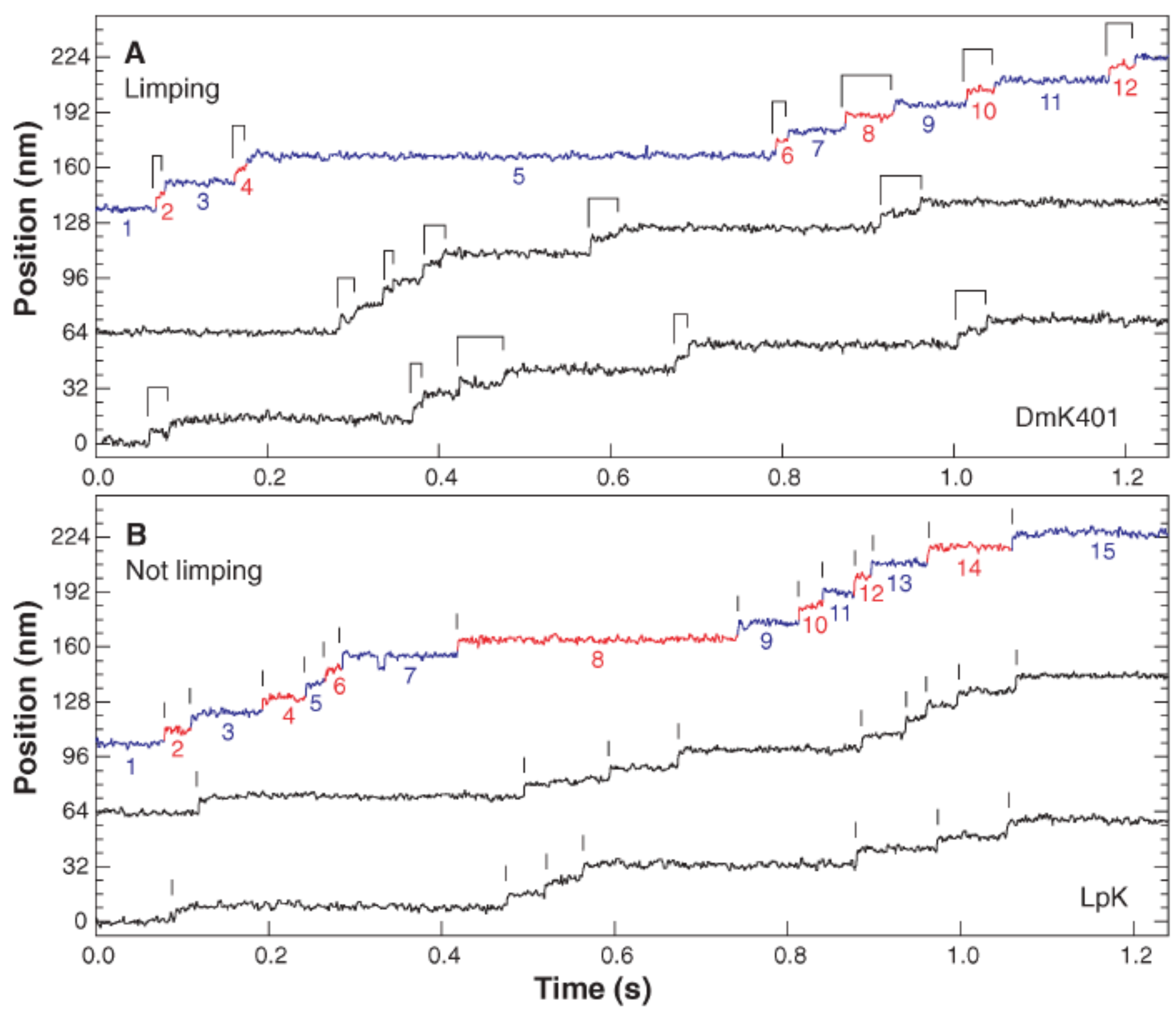


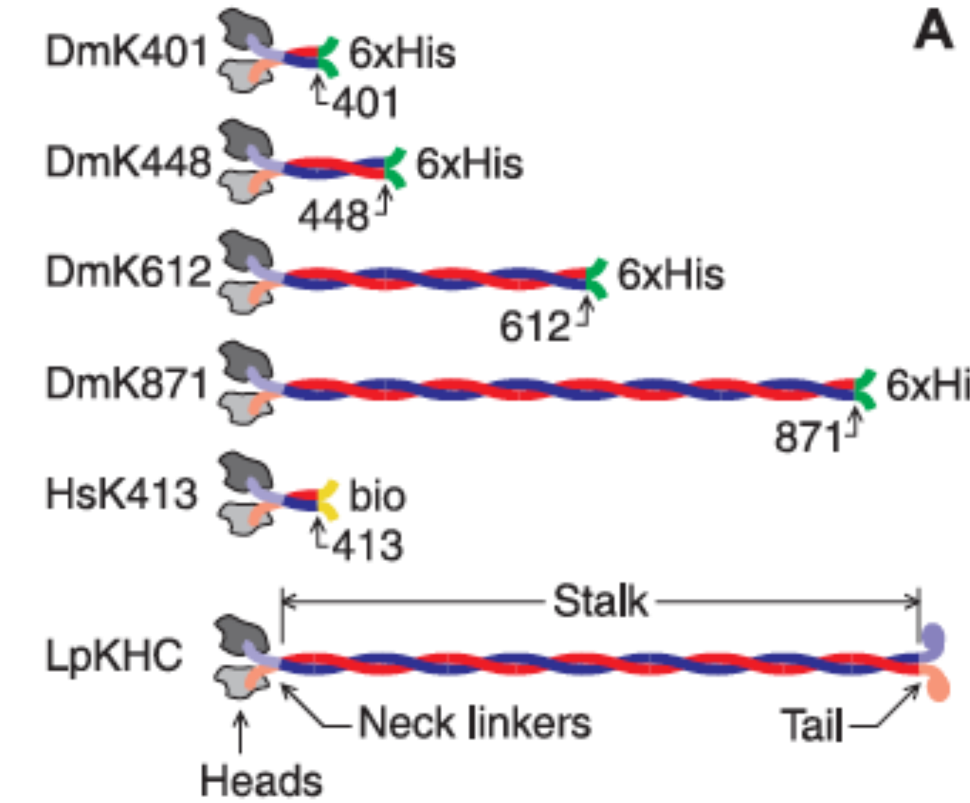
MP Sheetz and coworkers Biophys. J. (1995) 69:2011-2023

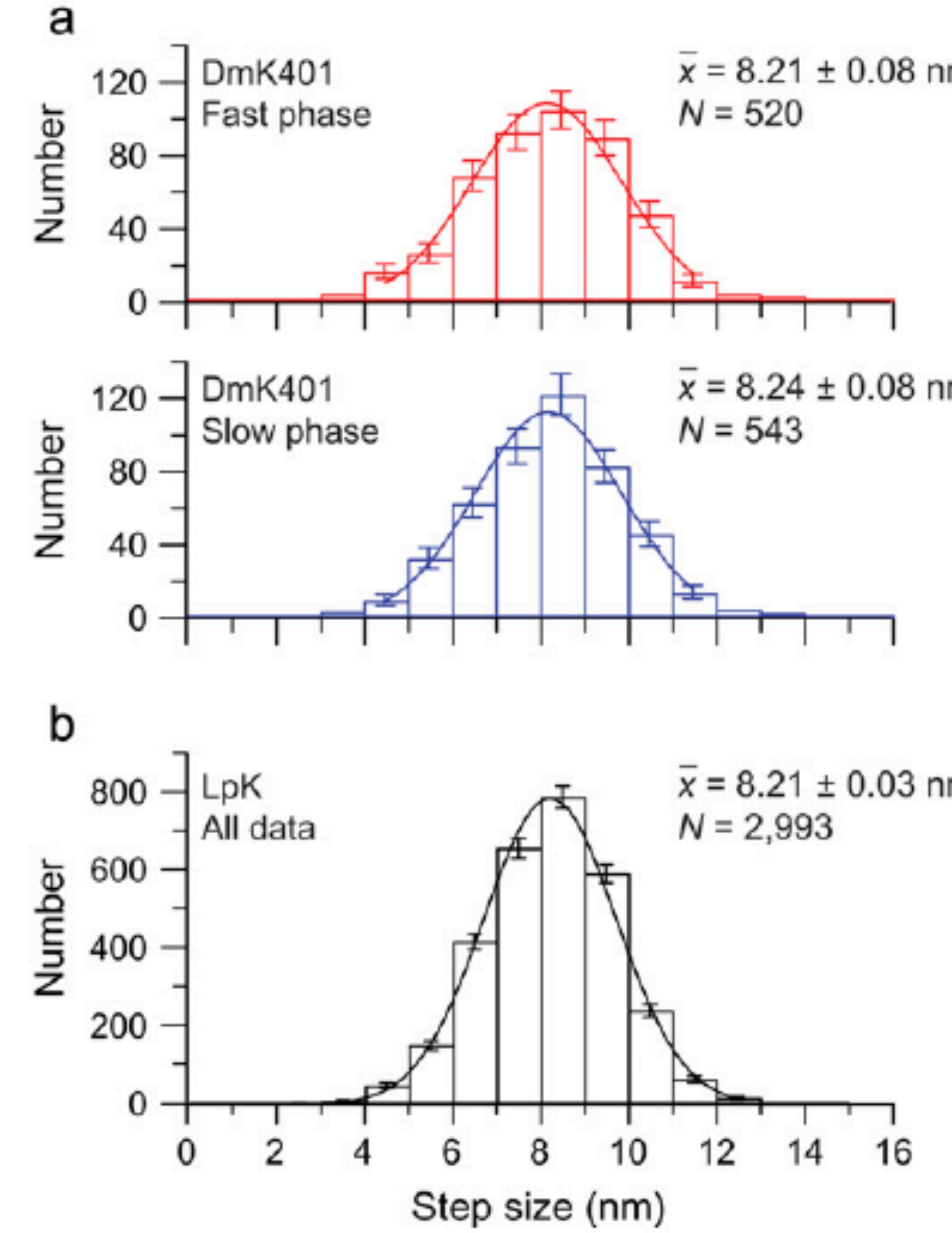


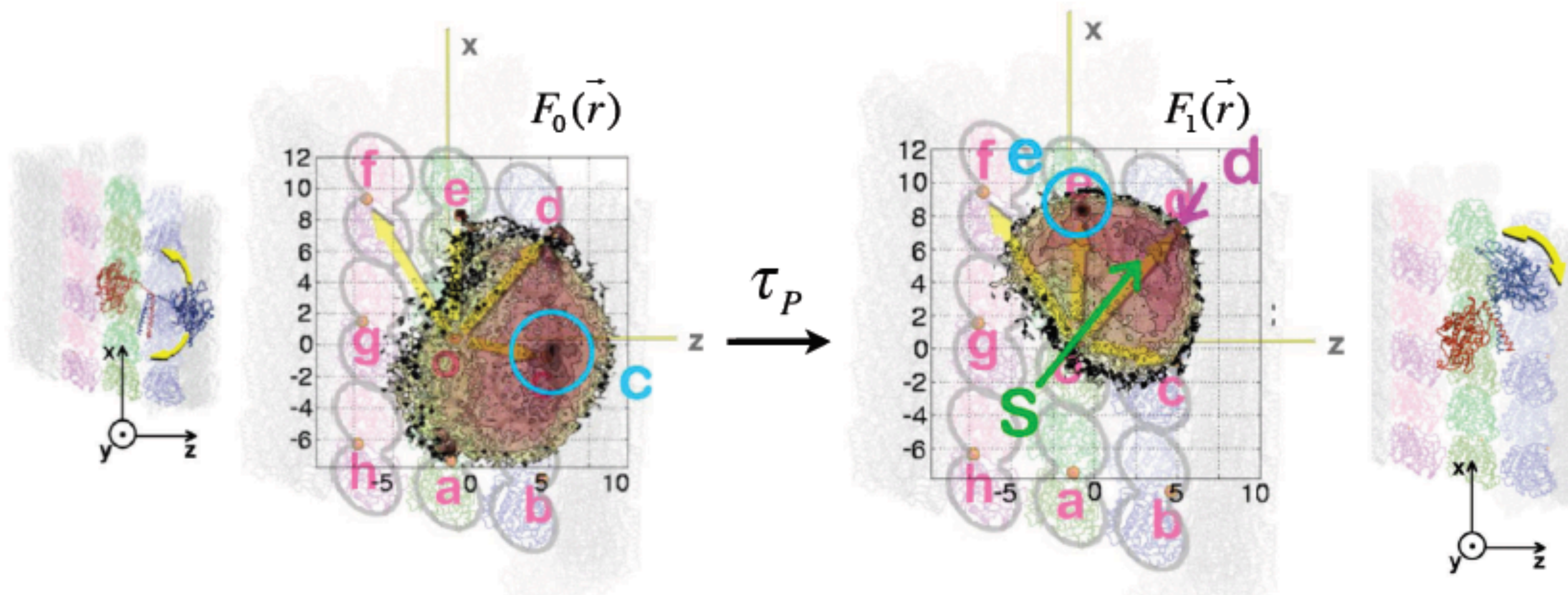
Block et al. PNAS (2003) 100:2351--2356


One-head-bound
Two-heads-bound
Tightrope
(8 / 8 / 8 / 8)
Straddle
(7 / 9 / 7 / 9)
Tightrope
(8 / 8 / 8 / 8)
Straddle
(8 / 8 / 8 / 8)
Asymmetric straddle
(7 / 9 / 7 / 9)
1 nm
+


A
Limping
DmK401
B
Not limping
LpK
Position (nm)
Time (s)


A
DmK401
6xHis
401
DmK448
448
DmK612
612
DmK871
871
HsK413
bio
413
LpKHC
Stalk
Neck linkers
Tail
Heads


a
DmK401
Fast phase
x̄ = 8.21 ± 0.08 nm
N = 520
DmK401
Slow phase
x̄ = 8.24 ± 0.08 nm
N = 543
b
LpK
All data
x̄ = 8.21 ± 0.03 nm
N = 2,993
Number
Step size (nm)



$F_0(\vec{r})$
τ_P
$F_1(\vec{r})$
x
z
y
a
b
c
d
e
f
g
h
o
S
12
10
8
6
4
2
0
-2
-4
-6
-5
5
10

Questions from Biophys. J. (2007) 92: 2986-2995

- Does kinesin take substeps? If so, over what time and distnace scales?
- What's the kinesin walking pattern, and what do we learn about its mechanics from this? (sym HoH, asym HoH, inchworm)
- How do the two kinesin heads mange to stay out of phase with one another during the stepping cycle (i.e., how are they "gated")?
- Where in the kinesin biochemical pathway is forward motion produced?
- Is the bacstepping cycle a reversal of the forward cycle, and does kinesin generate ATP under super-stall loads that force it to move backward?

Questions from Biophys. J. (2007) 92: 2986-2995

- Conversely, when kinesin is sped up by an assisting force, is it going through its normal biochemical cycle or by some other pathway ?
- When stepping processively, does kinesin spend most of its time in a two-heads bound (2HB) state or a one-head bound (1HB) state?
- Is the head-neck linker docking model correct (and does it suffice to explain actual stepping)? Does kinesin undertake a conformational "power stroke", or something like it (and if so, how large is it)?
- How does kinesin manage to track parallel to a single protofilament of the microtubule?